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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵: C07K 3/00, 17/06, A61K 39/00, 43/00, 49/00	A1	(11) International Publication Number: WO 94/29333 (43) International Publication Date: 22 December 1994 (22.12.94)
(21) International Application Number: PCT/US94/06424 (22) International Filing Date: 7 June 1994 (07.06.94) (30) Priority Data: 08/073,198 7 June 1993 (07.06.93) US (71) Applicant (for all designated States except US): BURROUGHS WELLCOME CO. [US/US]; 3030 Cornwallis Road, Re- search Triangle Park, NC 27709 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): BLACK, Christopher, D., V. [GB/US]; 2815 Aquarius Avenue, Silver Springs, MD 20906 (US). SNOW, Robert, Allen [CA/US]; 118 Cratin Lane, West Chester, PA 19380 (US). (74) Agents: NORTHRUP, Thomas, E.; Dressler, Goldsmith, Shore & Milnamow, Ltd., Two Prudential Plaza, Suite 4700, Chicago, IL 60601 (US) et al.	(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>	
(54) Title: IMMUNOREACTIVE REAGENTS EMPLOYING MONOAMINE OXIDASE (57) Abstract In one aspect this invention describes a non radioactive targeting immunoreagent comprising the residue of a proteinaceous active site of a monoamine oxidase enzyme (MAO), a linking group, and the residue of an immunoreactive material together with a radioactive delivery agent comprising a ligand specific for said MAO receptor moiety, a linking group, and a radioactive agent. In another aspect, this invention describes a non radioactive targeting immunoreagent comprising the residue of a ligand specific for an MAO proteinaceous active site receptor moiety, a linking group, and the residue of an immunoreactive material together with a radioactive delivery agent comprising the residue of an MAO proteinaceous active site receptor moiety, a linking group, and a radioactive agent. These compositions comprise useful systems for the production of an amplification of delivery of the radioactive agent to tumor sites in the therapy and diagnostic imaging of cancer.		

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IMMUNOREACTIVE REAGENTS EMPLOYING
MONOAMINE OXIDASEField of the Invention

5 This invention relates to the therapeutic
treatment and diagnostic imaging of cancer by means of a
tumor targeted sequential delivery system comprising a
primary non radioactive targeting immunoreagent and a
secondary radioactive delivery agent.

10

Background of the Invention

 The various, currently available, radiolabeled
immunoreactive proteins and methods which are employed
in diagnostic imaging and targeted therapeutic
15 applications suffer from certain disadvantages. For
example, radioimmunotherapy and diagnostic imaging with
the various currently available radiopharmaceuticals
which include radionuclide-containing immunoreactive
proteins can be less than optimal because these
20 radiopharmaceuticals may bind to non-target normal
tissue. This binding can result in undesirable toxicity
to normal tissue during therapeutic applications as well
as in high background signals during diagnostic imaging
applications; the radioactive component may then deposit
25 in healthy tissue. Also, long plasma half-lives of
currently available radionuclide-containing
immunoreactive proteins and slow clearance of
radionuclide from the body can result in prolonged

exposure of normal tissue to damaging effects of radiation and can produce unacceptable toxic effects in otherwise normal and disease free tissues in the body, especially in those tissues and cells most sensitive to radiation damage, e.g., the stem cells of the bone marrow and gastrointestinal tract. While the number of ionic radionuclides that can be associated with an immunoreactive protein is restricted by the number of sites of chelation available, an increase in that number which can be achieved by increasing the number of chelating agents conjugated to the protein can produce a decrease in the immunoreactivity of the protein. This can limit the number of such chelating agents that can be attached to the protein. The number of chelating agents that can be attached to an immunoreactive protein is also limited by the number of available groups such as, for example, amino groups suitable for use in attachment of the chelating agents and by the potential immunogenicity of the thus modified protein which, being highly derivatized, could be recognized by a host immune system as being haptenated.

It is an object of the present invention to overcome some of the aforementioned disadvantages of the currently available radiolabeled immunoreactive proteins.

R. R. Rando (*Molecular Pharmacology*, 1977, 13, 726-734) has reported that the para-substituted

fluorescent pargyline derivative, 5'-(N-dansyl)cadaveryl-p-carboxymethylpargyline, as well as analogous fluorescein and rhodamine derivatives can be used to specifically and irreversibly label isolated
5 mitochondrial monoamine oxidase. The dansyl group does not fit into the active site region of the enzyme but resides in a more polar environment and has rotational degrees of freedom independent of the macromolecule. It is concluded that "the fluorescent probe moiety of the
10 inhibitor does not bind specifically to the active site region but hangs more or less freely from the enzyme."

J. S. Fowler et al. in United States Patent Application A6052921 (1989) reveal a strategy for imaging and mapping the regional distribution of enzymes
15 in a living body by using positron emitter-labeled suicide enzyme inhibitors which bind irreversibly to the enzyme through catalysis, thereby labeling the enzyme. Carbon-11 labeled clorgyline and L-deprenyl are reported as selective probes for monoamine oxidase localization
20 and reactivity *in vivo* using positron emission tomography.

Summary of the Invention

In one embodiment, the present invention is directed to a non radioactive targeting immunoreagent (sometimes hereinafter referred to as NRTIR) comprising the residue of a monoamine oxidase, a linking group, and the residue of an immunoreactive material, which immunoreactive material can bind to sites on cells of a tissue of interest.

The present invention is also directed to a radioactive delivery agent (sometimes hereinafter referred to as RDA) comprising a ligand specific for said monoamine oxidase, a linking group, and a radioactive agent that is administered to the environs of said tissue. The ligand of said RDA will bind to the receptor of said NRTIR which is bound to the cells of the tissue of interest and thus provides an effective amount of radioactivity to said tissue. Unbound RDA can be removed rapidly from the environs of said tissue.

In particular, in one aspect (sometimes hereinafter referred to as System A), the present invention comprises an NRTIR comprising the residue of a receptor moiety which receptor moiety comprises the residue of a proteinaceous active site of a monoamine oxidase enzyme (sometimes hereinafter referred to as MAO), a linking group, and the residue of an immunoreactive material and an RDA comprising a ligand specific for said MAO receptor moiety, a linking group,

and a radioactive agent. In another aspect, (sometimes hereinafter referred to as System B), the present invention comprises a NRTIR comprising the residue of a ligand specific for an MAO receptor moiety, a linking group, and the residue of an immunoreactive material and an RDA comprising the residue of an MAO receptor moiety, a linking group, and a radioactive agent.

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Preferably, in System A, the present invention is directed to an NRTIR comprising the proteinaceous active site of a monoamine oxidase enzyme, a linking group, and the residue of an immunoreactive material such as a tumor targeting antibody together with an RDA comprising a ligand specific for said MAO receptor moiety, a linking group, and a radioactive agent comprising a chelating agent and a radionuclide.

20
Preferably, in System B, the present invention is directed to a NRTIR comprising the residue of a ligand specific for an MAO receptor moiety, a linking group, and the residue of an immunoreactive material such as a tumor targeting antibody together with an RDA comprising the residue of an MAO receptor moiety, a linking group, and a radioactive agent comprising a chelating agent and a radionuclide.

25
The present invention is also directed to pharmaceutical and diagnostic compositions that contain the NRTIR and a pharmaceutically acceptable carrier, and to pharmaceutical and diagnostic compositions that

contain the RDA and a pharmaceutically acceptable carrier.

The present invention is further directed to diagnostic imaging and therapeutic methods comprising sequentially administering an effective amount of NRTIR to a patient in need of such diagnosis or treatment, allowing the NRTIR to bind to sites on cells of a tissue of interest, cleaning unbound NRTIR from the environs of said tissue, and administering RDA to said patient.

In addition to recognizing and binding to the residue of the receptor of the NRTIR, chief characteristics of the RDA in these systems are:

a) for a given degree of NRTIR immunoreactive protein modification, the RDA contains multiple chelating agents which in total are capable of binding more ions of a radiometal per molecule of immunoreactive protein than can be bound per molecule by direct metallation of a previously available immunoreactive protein-chelating agent conjugate;

b) the interaction of the receptor of the NRTIR with the ligand of the RDA should be as long-lasting as possible (i.e., having a high affinity for each other, preferably undergoing effectively irreversible binding) so that loss of the RDA to other sites after binding is minimized; and

c) any RDA which fails to bind to the receptor of the tissue bound NRTIR is rapidly removed, for

example, from the plasma preferably by excretion from the body.

In one aspect, the present invention comprises an antigen on the surface of a cell, preferably a tumor cell, to which is bound a single molecule of an immunoreactive protein of the NRTIR. To the immunoreactive protein of the NRTIR are attached multiple copies (n) of a receptor, each of which binds a ligand of the RDA containing multiple chelator sites (m) for metal sequestration. The total number of radiometal atoms capable of being bound per antigen is then the product of (n) multiplied by (m). This is in contrast with the binding of a previously available immunoreactive protein containing multiple copies (c) of a chelator where the value of (c) is restricted by the number of conjugations that can be performed while retaining the immunoreactivity of the protein for the antigen. Because of the above limiting restriction on the degree of modification, the value of (n) will be approximately the same as the value of (c) so that the delivery system of the present invention will augment the maximum number of radiometal atoms bound per cell antigen by a factor of (m).

Description of Preferred Embodiments

In preferred embodiments, the above-described
 non radioactive targeting immunoreagent (NRTIR) and
 radioactive delivery agent (RDA) comprise moieties
 represented in System A (4 systems) and System B below:

SYSTEM A

Non Radioactive

Targeting

ImmunoReagent

NRTIR

Radioactive Delivery

Agent

RDA

(1) Immunoreactive group

(+ linking group

+ receptor)_n

Ligand

(+ linking group

+ chelating agent

+ radionuclide)_m(2) Z-(L₁-Rec)_nD-(L₂-Q-M)_m(3) Z-(L₁-Rec)_nClorgyline-(L₂-Q-M)_m(4) Z-(L₁-Rec)_nPargylinyl-(L₂-Q-M)_m

SYSTEM B

Non Radioactive

Targeting

ImmunoReagent

Radioactive Delivery Agent

NRTIR

RDA

(1) Immunoreactive group

(+ linking group

+ ligand)_n

Receptor

(+ linking group

+ chelating agent

+ radionuclide)_m(2) Z-(L₁-MAO ligand)_nRec-(L₂-Q-M)_m(3) Z-(L₁-CLO)_nRec-(L₂-Q-M)_m(4) Z-(L₁-PARG)_nRec-(L₂-Q-M)_m

wherein:

Z is the residue of an immunoreactive group;

Rec is the residue of an MAO;

D is the residue of a ligand that will bind to the MAO
receptor;MAO ligand is the residue of a ligand that will bind to
an MAO active site;

CLO is the residue of a clorgyline analog;

PARG is the residue of a pargylinyl analog;

L₁ and L₂ are independently the residues of a linking group that may independently contain a spacing group;
Q is the residue of a chelating group;
M is a radionuclide; and
5 n and m are independently integers greater than zero.

Preferred embodiments of these materials are further described below.

10 a) Immunoreactive group Z

The immunoreactive group, Z, can be selected from a wide variety of naturally occurring or synthetically prepared materials. Z preferably is an antibody or antibody fragment which recognizes and is specific for a
15 tumor associated antigen. In some embodiments, Z can contain an immunoreactive group covalently bonded thereto through a chemical bond or a linking group derived from the residue of a protein reactive group and the residue of a reactive group on the protein. As used
20 herein, the term "immunoreactive protein" which can be abbreviated by "IRP" also includes an organic compound which is capable of covalently bonding to the protein and which is found in a living organism or is useful in the diagnosis, treatment or genetic engineering of
25 cellular material or living organisms, and which has a capacity for interaction with another component which

may be found in biological fluids or associated with cells to be treated such as tumor cells.

The immunoreactive group can be selected from a wide variety of naturally occurring or synthetically prepared materials, including, but not limited to enzymes, amino acids, peptides, polypeptides, proteins, lipoproteins, glycoproteins, lipids, phospholipids, hormones, growth factors, steroids, vitamins, polysaccharides, viruses, protozoa, fungi, parasites, rickettsia, molds, and components thereof, blood components, tissue and organ components, pharmaceuticals, haptens, lectins, toxins, nucleic acids (including oligonucleotides), antibodies (monoclonal and polyclonal), anti-antibodies, antibody fragments, antigenic materials (including proteins and carbohydrates), avidin and derivatives thereof, biotin and derivatives thereof, and others known to one skilled in the art. In addition, an immunoreactive group can be any substance which when presented to an immunocompetent host will result in the production of a specific antibody capable of binding with that substance, or the antibody so produced, which participates in an antigen-antibody reaction.

Preferred immunoreactive groups are antibodies and various immunoreactive fragments thereof, as long as they contain at least one reactive site for reaction with a protein reactive group as described herein. That

site can be inherent to the immunoreactive species or it can be introduced through appropriate chemical modification of the immunoreactive species. In addition to antibodies produced by the techniques outlined above, other antibodies and proteins produced by the techniques of molecular biology are specifically included.

Preferably, the immunoreactive group does not bind in an immunoreactive sense to the residue of a monoamine oxidase active site or to the residue of a ligand that has an affinity for a monoamine oxidase active site so as to inhibit binding between the two species in Systems A and B.

As used herein, the term "antibody fragment" refers to an immunoreactive material which comprises a residue of an antibody, which antibody characteristically exhibits an affinity for binding to an antigen. The term affinity for binding to an antigen, as used herein, refers to the thermodynamic expression of the strength of interaction or binding between an antibody combining site and an antigenic determinant and, thus, of the stereochemical compatibility between them. As such, it is the expression of the equilibrium or association constant for the antibody-antigen interaction. The term "affinity" as used herein also refers to the thermodynamic expression of the strength of interaction or binding between a ligand and a receptor and, thus, of the stereochemical compatibility between them. As such,

it is the expression of the equilibrium or association constant for the ligand-receptor interaction.

With respect to the affinity of binding of an antibody to an antigen, antibody fragments exhibit a percentage of said affinity for binding to said antigen, that percentage being in the range of 0.001 percent to 1,000 percent, preferably 0.01 percent to 1,000 percent, more preferably 0.1 percent to 1,000 percent, and most preferably 1.0 percent to 100 percent, of the relative affinity of said antibody for binding to said antigen.

An antibody fragment can be produced from an antibody by a chemical reaction comprising one or more chemical bond cleaving reactions; by a chemical reaction comprising of one or more chemical bond forming reactions employing as reactants one or more chemical components selected from a group comprising amino acids, peptides, carbohydrates, linking groups as defined herein, spacing groups as defined herein, protein reactive groups as defined herein, and antibody fragments such as are produced as described herein and by a molecular biological process, a bacterial process, or by a process comprising or resulting from the genetic engineering of antibody genes.

An antibody fragment can be derived from an antibody by a chemical reaction comprising one or more of the following reactions:

(a) cleavage of one or more chemical bonds of which an antibody is comprised, said bonds being selected from, for example, carbon-nitrogen bonds, sulfur-sulfur bonds, carbon-carbon bonds, carbon-sulfur bonds, and carbon-oxygen bonds, and wherein the method of said cleavage is selected from:

(i) a catalysed chemical reaction comprising the action of a biochemical catalyst such as an enzyme such as papain or pepsin which enzymes to those skilled in the art are known to produce antibody fragments commonly referred to as Fab and Fab'2, respectively;

(ii) a catalysed chemical reaction comprising the action of an electrophilic chemical catalyst such as a hydronium ion which, for example, favorably occurs at a pH equal to or less than 7;

(iii) a catalysed chemical reaction comprising the action of a nucleophilic catalyst such as a hydroxide ion which, for example, favorably occurs at a pH equal to or greater than 7;

(iv) a chemical reaction comprising a substitution reaction employing a reagent such which is consumed in a stoichiometric manner such as, for example, a substitution reaction at a sulfur atom of a disulfide bond by a reagent comprising a sulfhydryl group (comprising a -SH group) or an anionic sulfide group (comprising an $-S^-$ group in the form of a salt such as a $-S^- Na^+$ group);

(v) a chemical reaction comprising a reduction reaction such as, for example, the reduction of a disulfide bond; and

(vi) a chemical reaction comprising an oxidation reaction such as the oxidation of a carbon-oxygen bond of a hydroxyl group or the oxidation of a carbon-carbon bond of a vicinal diol group such as occurs in a carbohydrate moiety; or

(b) formation of one or more chemical bonds between one or more reactants, such as formation of one or more covalent bonds selected from, for example, carbon-nitrogen bonds (such as, for example, amide bonds, amine bonds, hydrazone bonds, imine bonds, and thiourea bonds), sulfur-sulfur bonds such as disulfide bonds, carbon-carbon bonds, carbon-sulfur bonds, and carbon-oxygen bonds, and employing as reactants in said chemical bond formation one or more reagents comprising amino acids, peptides, carbohydrates, linking groups as defined herein, spacing groups as defined herein, protein reactive groups as defined herein, and antibody fragments such as are produced as described in (a), above; or

(c) an antibody fragment can be derived by formation of one or more non-covalent bonds between one or more reactants. Such non-covalent bonds comprise hydrophobic interactions such as occur in an aqueous medium between chemical species that independently

comprise mutually accessible regions of low polarity such as regions comprising aliphatic and carbocyclic groups, and of hydrogen bond interactions such as occur in the binding of an oligonucleotide with a complementary oligonucleotide; or

(d) an antibody fragment can be produced as a result of the methods of molecular biology or by genetic engineering of antibody genes, for example, in the genetic engineering of a single chain immunoreactive group or a Fv fragment.

An antibody fragment can be produced as a result of a combination of one or more of the above methods.

In certain embodiments, the immunoreactive group can be an enzyme which has a reactive group for attachment to the residue of a monoamine oxidase active site in System A or to the residue of a ligand that has an affinity for binding to such a site in System B by means of a linking group L_z .

Representative enzymes include, but are not limited to, aspartate aminotransaminase, alanine aminotransaminase, lactate dehydrogenase, creatine phosphokinase, gamma glutamyl transferase, alkaline acid phosphatase, prostatic acid phosphatase, horseradish peroxidase and various esterases.

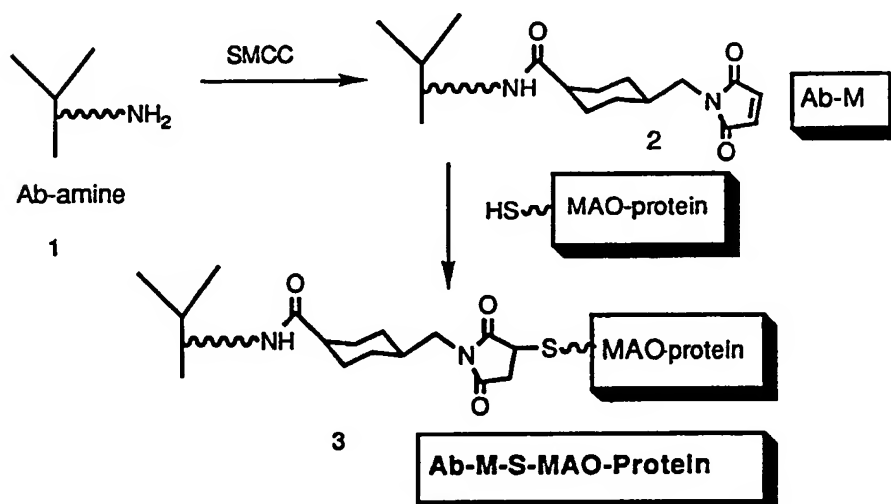
If desired, the immunoreactive group can be modified or chemically altered to provide a reactive group for use in the attachment to the residue of a

monoamine oxidase active site in System A or to the residue of a ligand that has an affinity for binding to such a site in System B through a linking group as described below by techniques known to those skilled in the art. Such techniques include the use of linking moieties and chemical modification such as described in WO-A-89/02931 and WO-A-89/2932, which are directed to modification of oligonucleotides, and U.S. Patent No. 4,719,182.

Two highly preferred uses for the compositions of this invention are for the diagnostic imaging of tumors and the radiological treatment of tumors. Preferred immunological groups therefore include antibodies to tumor-associated antigens. An antibody is sometimes hereinafter referred to as Ab. Specific non-limiting examples of antibodies include B72.3 and related antibodies (described in U.S. Patent Nos. 4,522,918 and 4,612,282) which recognize colorectal tumors; 9.2.27 and related anti-melanoma antibodies; D612 and related antibodies which recognize colorectal tumors; UJ13A and related antibodies which recognize small cell lung carcinomas; NRLU-10, NRCO-02 and related antibodies which recognize small cell lung carcinomas and colorectal tumors (Pan-carcinoma); 7E11C5 and related antibodies which recognize prostate tumors; CC49 and related antibodies which recognize colorectal tumors; TNT and related antibodies which recognize necrotic

tissue; PR1A3 and related antibodies which recognize colon carcinoma; ING-1 and related antibodies, which are described in International Patent Publication WO-A-90/02569; B174, C174 and related antibodies which recognize squamous cell carcinomas; B43 and related antibodies which are reactive with certain lymphomas and leukemias; and anti-HLB and related monoclonal antibodies. An especially preferred antibody is ING-1.

Scheme 1



b) Receptor

Preferred receptors comprise the residue of a monoamine oxidase (MAO; monoamine:oxygen oxidoreductase, EC 1.4.3.4) active site. The MAO active site can comprise any MAO enzyme, in whole or in part, isolated from any source or modified by well known techniques of molecular biology as long as it maintains MAO activity.

MAO exists as two isozymes, MAO-A and MAO-B. The tissue distribution of these isozymes is different, with MAO-A being found in its purest form in the human placenta, and MAO-B found in essentially pure form in human blood platelets and predominantly in the brain. The MAO isozyme chosen for use in the compositions of the present invention depends on the degrees of specificity desired. For example, choosing an inhibitor of MAO-A avoids potential toxicity in platelets or the brain where MAO-B predominates, but may have a higher toxicity to the liver where MAO-A is the major isotype. Preferably, the MAO is a recombinant human enzyme. Ultimately, in System A, the active site of the MAO is genetically engineered into a recombinant human matrix form while the specificity of the enzyme active site for MAO substrate analogs is maintained.

In system A, the MAO is covalently coupled, i.e., conjugated, to an immunoreactive group, preferably an antibody or an antibody fragment, most preferably to ING-1, to form the NRTIR (i.e., Z-L₁-Rec) of the system.

In system B, in one embodiment, the MAO as a component of a radioactive delivery agent [i.e., an RDA, Rec-(L₂-Q-M)_m] is attached to one or more chelating groups, each by means of a linking group, and the chelating group is associated with a radionuclide. Preferably the chelating group is TMT, the linking group is as described below, and the radionuclide is ⁹⁰Y. In

another embodiment, the RDA comprises an MAO that contains one or more radionuclides that are covalently attached, either directly to one or more components of the MAO or to one or more components that are attached by a linking group as described below to the MAO. Preferably, said covalently attached radionuclide is a radioisotope of iodine attached to an aromatic ring containing moiety.

In System A, chemical conjugation can be achieved, for example, by a technique comprising the use of a linking group (L₁) which is introduced through modification of, for example, a site on an immunoreactive group. The introduction of activated groups such as activated ethylene groups (e.g., maleimide groups) on to amine groups such as lysine epsilon-amines of a protein is represented in Scheme 1. Other techniques include the use of heterobifunctional linking moieties and chemical modifications such as the examples described in U. S. Patent No. 4,719,182. Additionally, those chemicals such as SMCC, i.e., succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate, which are commonly commercially available, for example, from Pierce Chemical Company are included as non-limiting examples.

In both System A and System B in one aspect, chemical conjugation is otherwise achieved by using a linking group (L₁ and L₂, respectively) which is

introduced through mild reduction of a disulfide bond in the MAO (or in the MAO modified by reagents which contain disulfide bonds, one non-limiting example of which is succinimidyl 3-(2-pyridyldithio)propionate, SPDP, available from Pierce Chemical Company) with a reducing reagent such as dithiothreitol to produce sulfhydryl (SH) sites in the reduced MAO protein moiety. In System A, addition of the thus reduced MAO protein moiety (HS-MAO protein) to the above described maleimide modified antibody (Ab-M) results in an antibody/receptor conjugate (Ab-M-S-MAO protein) linked together by one or more thioether bonds. Additionally, those chemicals which are commonly commercially available, for example, from Pierce Chemical Company and the like which are useful in the covalent attachment of two proteins are included as non-limiting examples in the coupling of MAO to antibody in System A.

In System B, addition of the thus reduced MAO protein moiety to a chelating agent which contains a precursor of a linking group comprising an activated ethylene group such as a maleimide group as described above results in a MAO/chelating agent conjugate linked together by a thioether bond. Similarly, addition of the thus reduced immunoreactive protein moiety to the residue of a ligand which contains a precursor of a linking group comprising an activated ethylene group such as a maleimide group results in a immunoreactive

protein moiety/ligand conjugate linked together by a thioether bond.

In System A, other groups are useful in the coupling together of two proteins such as the immunoreactive material to the receptor moiety, particularly if the above reagents are utilized. Suitable reactive sites on the immunoreactive material and on the receptor moiety include:

amine sites of lysine;

terminal peptide amines;

carboxylic acid sites, such as are available in aspartic acid and glutamic acid;

sulfhydryl sites;

carbohydrate sites;

activated carbon-hydrogen and carbon-carbon bonds which can react through insertion via free radical reaction or nitrene or carbene reaction of a so activated residue;

sites of oxidation;

sites of reduction;

aromatic sites such as tyrosine; and

hydroxyl sites.

In System A, the ratio of MAO to immunoreactive group such as an antibody can vary widely from about 0.5 to 10 or more. In bulk, mixtures comprised of immunoreactive groups which are unmodified and immunoreactive groups which are modified with MAO are also suitable. Such mixtures can have a bulk ratio of

MAO to immunoreactive group of from about 0.1 to about 10.

5 In System A, in preferred embodiments, the mole ratio of MAO to immunoreactive group is from about 1:1 to about 6:1. It is specifically contemplated that with knowledge of the DNA sequence that encodes MAO, especially human MAO, a fusion protein can be made between the antibody and the MAO, or portions thereof, through the use of genetic engineering techniques. It is specifically contemplated that in all of these
10 compositions of MAO bound to antibody, MAO retains a capacity to bind to the ligands described in the invention.

15 In System B, the ratio of ligand to immunoreactive group such as an antibody can vary widely from about 0.5 to 10 or more. In bulk, mixtures comprised of immunoreactive groups which are unmodified and immunoreactive groups which are modified with ligand are also suitable. Such mixtures can have a bulk ratio of
20 ligand to immunoreactive group of from about 0.1 to about 10. In preferred embodiments, the mole ratio of ligand to immunoreactive group is from about 1:1 to about 6:1.

25 In System A, following the reaction of the immunoreactive group, preferably of an antibody or an antibody fragment, to MAO, the conjugate is purified by passage of the material through a gel permeation column

such as Superose 6 using an appropriate elution buffer or by elution from a HPLC column such as a Shodex WS-803F size exclusion column. Both these methods separate the applied materials by molecular size resulting in the elution of the antibody/MAO conjugate in a different fraction from any residual non-conjugated MAO.

In System A, the concentrations of the antibody in the conjugate solutions are determined by the BioRad protein assay using bovine immunoglobulin as the protein standard.

In System B, following the reaction of the immunoreactive group, preferably of an antibody or an antibody fragment, to the residue of a ligand, the conjugate is purified by passage of the material through a gel permeation column such as Superose 6 using an appropriate elution buffer or by elution from a HPLC column such as a Shodex WS-803F size exclusion column. Both these methods separate the applied materials by molecular size resulting in the elution of the antibody/ligand conjugate in a different fraction from any residual non-conjugated ligand.

In System B, the concentrations of the antibody in the conjugate solutions are determined by the BioRad protein assay using bovine immunoglobulin as the protein standard.

In System A, the ability of the antibody to bind to its target antigen following conjugation to MAO can be

assayed by ELISA or flow cytometry. A 30 cm x 7.5 mm TSK-G3000SW size-exclusion HPLC column (Supelco) fitted with a guard column of the same material can be used to determine the amount of aggregation in the final conjugate.

In System B, the ability of the antibody to bind to its target antigen following conjugation to the residue of a ligand can be assayed by ELISA or flow cytometry. A 30 cm x 7.5 mm TSK-G3000SW size-exclusion HPLC column (Supelco) fitted with a guard column of the same material can be used to determine the amount of aggregation in the final conjugate.

In System A, the monoamine oxidase enzymic activity of the antibody-associated MAO can be assayed by following the deamination of biogenic monoamines (e.g., 5-hydroxytryptamine, dopamine, norepinephrine, and dietary tryamine) by MAO. An especially useful method for assaying monoamine oxidase enzymic activity involves following the rate of oxidation of kynurine to 4-hydroxyquinoline by MAO as described by Weyler, W. and Salach, J.I. (*J. Biological Chemistry*, 260:13199 - 13207 [1985]). This method can also be used to assay the MAO inhibitory effects of the novel MAO binding ligands which are modified to include chelating agents as described in this invention.

In System B, the monoamine oxidase enzymic activity of the chelating agent-associated MAO can be assayed by

following the rate of oxidation of kynurine to 4-hydroxyquinoline as described by Weyler, W. and Salach, J.I. (*J. Biological Chemistry*, 260:13199 - 13207 [1985]). This method can also be used to assay the MAO inhibitory effects of the novel ligands which have an affinity for binding to MAO and which are linked to immunoreactive groups as described in this invention.

c) Linking Group

L_1 and L_2 in System A and System B are independently a chemical bond or the residue of a linking group. In one aspect, the phrase "residue of a linking group" as used herein refers to a moiety that remains, results, or is derived from the reaction of a protein reactive group with a reactive site on a protein. The phrase "protein reactive group" as used herein refers to any group which can react with functional groups typically found on proteins. However, it is specifically contemplated that such protein reactive groups can also react with functional groups typically found on relevant nonprotein molecules. Thus, the linking groups useful in the practice of this invention derive from those groups which can react with any relevant molecule "Z" or "Rec" as described above containing a reactive group, whether or not such relevant molecule is a protein, to form a linking group. In one aspect, preferred linking groups thus formed

include the linking group, L₁, between the immunoreactive group, "Z", and the MAO active site containing species, "Rec", in the NRTIR System A; the linking group, L₁, between the immunoreactive group, "Z", and MAO ligand species (e.g., "CLO" or "PARG") in the NRTIR in System B; and the linking group, L₂, between the MAO active site containing species, "Rec", and the chelating agent, "Q", in the RDA in System B; and between the MAO ligand species (e.g., "CLO" or "PARG") and the chelating agent, "Q", in the RDA in System A.

Preferred linking groups are derived from protein reactive groups selected from but not limited to:

(1) a group that will react directly with amine, alcohol, or sulfhydryl groups on the immunoreactive protein or biological molecule containing the reactive group, for example, active halogen containing groups including, for example, chloromethylphenyl groups and chloroacetyl [ClCH₂C(=O)-] groups, activated 2-(leaving group substituted)-ethylsulfonyl and ethylcarbonyl groups such as 2-chloroethylsulfonyl and 2-chloroethylcarbonyl; vinylsulfonyl; vinylcarbonyl; epoxy; isocyanato; isothiocyanato; aldehyde; aziridino; succinimidoxycarbonyl; activated acyl groups such as carboxylic acid halides; mixed anhydrides and the like; and other groups known to be useful in conventional photographic gelatin hardening agents;

(2) a group that can react readily with modified proteins or biological molecules containing the immunoreactive group, i.e., proteins or biological molecules containing the immunoreactive group modified to contain reactive groups such as those mentioned in (1) above, for example, by oxidation of the protein to an aldehyde or a carboxylic acid, in which case the "linking group" can be derived from protein reactive groups selected from amino, alkylamino, arylamino, hydrazino, alkylhydrazino, arylhydrazino, carbazido, semicarbazido, thiocarbazido, thiosemicarbazido, sulfhydryl, sulfhydrylalkyl, sulfhydrylaryl, hydroxy, carboxy, carboxyalkyl and carboxyaryl. The alkyl portions of said linking groups can contain from 1 to about 20 carbon atoms. The aryl portions of said linking groups can contain from about 6 to about 20 carbon atoms; and

(3) a group that can be linked to the protein or biological molecule containing the immunoreactive group, or to the modified protein as noted in (1) and (2) above by use of a crosslinking agent. The residues of certain useful crosslinking agents, such as, for example, homobifunctional and heterobifunctional gelatin hardeners, bisepoxides, and bisisocyanates can become a part of, i.e., a linking group in, for example, the protein-(MAO active site-containing species) conjugate in System A during the crosslinking reaction. Other

useful crosslinking agents, however, can facilitate the crosslinking, for example, as consumable catalysts, and are not present in the final conjugate. Examples of such crosslinking agents are carbodiimide and carbamoylonyl crosslinking agents as disclosed in U.S. Patent No. 4,421,847 and the ethers of U.S. Patent No. 4,877,724. With these crosslinking agents, one of the reactants such as the immunoreactive group must have a carboxyl group and the other such as the oligonucleotide containing species must have a reactive amine, alcohol, or sulfhydryl group. In amide bond formation, the crosslinking agent first reacts selectively with the carboxyl group, then is split out during reaction of the thus "activated" carboxyl group with an amine to form an amide linkage between, for example, the protein and MAO active site containing species, thus covalently bonding the two moieties. An advantage of this approach is that crosslinking of like molecules, e.g., proteins with proteins or MAO active site containing species with themselves is avoided, whereas the reaction of, for example, homo-bifunctional crosslinking agents is nonselective and unwanted crosslinked molecules are obtained.

Preferred useful linking groups are derived from various heterobifunctional cross-linking reagents such as those listed in the Pierce Chemical Company Immunotechnology Catalog - Protein Modification Section,

(1991 and 1992). Useful non-limiting examples of such reagents include:

5 Sulfo-SMCC Sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate.

10 Sulfo-SIAB Sulfosuccinimidyl (4-iodoacetyl)aminobenzoate.

Sulfo-SMPB Sulfosuccinimidyl 4-(p-maleimidophenyl)butyrate.

15 2-IT 2-Iminothiolane.

SATA N-Succinimidyl S-acetylthioacetate.

20 In addition to the foregoing description, the linking groups, in whole or in part, can also comprise and be derived from complementary sequences of nucleotides and residues of nucleotides, both naturally occurring and modified, preferably non-self-associating oligonucleotide sequences. Particularly useful, non-limiting reagents for incorporation of modified
25 nucleotide moieties containing reactive functional groups, such as amine and sulfhydryl groups, into an oligonucleotide sequence are commercially available

from, for example, Clontech Laboratories Inc. (Palo Alto California) and include Uni-Link AminoModifier (Catalog # 5190), Biotin-ON phosphoramidite (Catalog # 5191), N-MMT-C6-AminoModifier (Catalog # 5202), AminoModifier II (Catalog # 5203), DMT-C6-3'Amine-ON (Catalog # 5222), C6-ThiolModifier (Catalog # 5211), and the like. In one aspect, linking groups of this invention are derived from the reaction of a reactive functional group such as an amine or sulfhydryl group as are available in the above Clontech reagents, one or more of which has been incorporated into an oligonucleotide sequence, with, for example, one or more of the previously described protein reactive groups such as heterobifunctional protein reactive groups, one or more of which has been incorporated into an immune reactive agent or MAO active site containing moiety of this invention.

In System A, the complementary oligonucleotide sequences are attached to two components of the conjugate, one sequence to the immune reactive agent and the complementary oligonucleotide sequence to the MAO active site containing moiety. The hybrid formed between the two complementary oligonucleotide sequences then comprises the linking group between the immune reactive agent and the MAO active site containing moiety.

In System B, the complementary oligonucleotide sequences are attached to two components of the

conjugate, one sequence to the residue comprising one or more chelating agents and the complementary oligonucleotide sequence to the MAO active site containing moiety. The hybrid formed between the two complementary oligonucleotide sequences then comprises the linking group between the MAO active site containing moiety and the chelating agent(s).

In System B, of course, two or more copies of the same oligonucleotide sequence can be linked, for example, in tandem to one MAO active site containing moiety and a complementary oligonucleotide sequence comprising multiple chelating agents can be added. The multiple hybrids formed between the two complementary oligonucleotide sequences then comprise the linking group between the MAO active site containing moiety and multiple chelating agents.

Likewise, in system B, one or more MAO active site binding ligands can be attached to the immunoreactive group using complementary oligonucleotide hybrids as described above.

In System A, analogously, multiple MAO sequences can be attached to the immunoreactive protein.

Likewise, in system A, one or more MAO active site binding ligands can be attached to multiple chelating agents using complementary oligonucleotide hybrids as described above.

d) Residues of Chelating groups

Q in Systems A and B represents the residues of chelating groups. The chelating groups of this invention can comprise the residue of one or more of a wide variety of chelating agents that can have a radionuclide associated therewith. As is well known, a chelating agent is a compound containing donor atoms that can combine by coordinate bonding with a metal atom to form a cyclic structure called a chelation complex or chelate. This class of compounds is described in the Kirk-Othmer Encyclopedia of Chemical Technology, Vol. 5, 339-368.

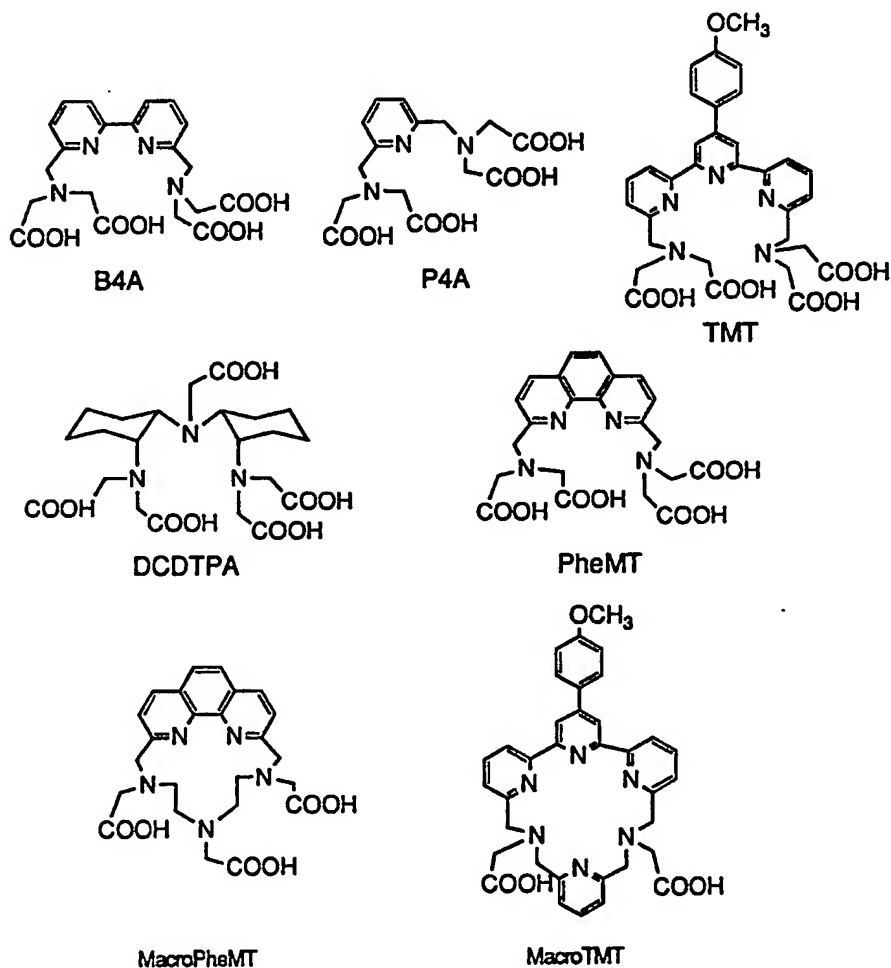
The residues of suitable chelating agents can be independently selected from polyphosphates, such as sodium tripolyphosphate and hexametaphosphoric acid; aminocarboxylic acids, such as ethylenediaminetetraacetic acid, N-(2-hydroxyethyl)ethylene-diaminetriacetic acid, nitrilotriacetic acid, N,N-di(2-hydroxyethyl)glycine, ethylenebis(hydroxyphenylglycine) and diethylenetriamine pentacetic acid; 1,3-diketones, such as acetylacetone, trifluoroacetylacetone, and thenoyltrifluoroacetone; hydroxycarboxylic acids, such as tartaric acid, citric acid, gluconic acid, and 5-sulfosalicylic acid; polyamines, such as ethylenediamine, diethylenetriamine, triethylenetetramine, and triaminotriethylamine; aminoalcohols, such as triethanolamine and N-(2-

hydroxyethyl)ethylenediamine; aromatic heterocyclic bases, such as 2,2'-dipyridyl, 2,2'-diimidazole, dipicoline amine and 1,10-phenanthroline; phenols, such as salicylaldehyde, disulfopyrocatechol, and
5 chromotropic acid; aminophenols, such as 8-hydroxyquinoline and oximesulfonic acid; oximes, such as dimethylglyoxime and salicylaldoxime; peptides containing proximal chelating functionality such as polycysteine, polyhistidine, polyaspartic acid,
10 polyglutamic acid, or combinations of such amino acids; Schiff bases, such as disalicylaldehyde 1,2-propylenediimine; tetrapyrroles, such as tetraphenylporphin and phthalocyanine; sulfur compounds, such as toluenedithiol, meso-2,3-dimercaptosuccinic
15 acid, dimercaptopropanol, thioglycolic acid, potassium ethyl xanthate, sodium diethyldithiocarbamate, dithizone, diethyl dithiophosphoric acid, and thiourea; synthetic macrocyclic compounds, such as dibenzo[18]crown-6, (CH₃)₆-[14]-4,11-diene-N₄, and
20 (2.2.2-cryptate); and phosphonic acids, such as nitrilotrimethylene-phosphonic acid, ethylenediaminetetra(methylenephosphonic acid), and hydroxyethylidenediphosphonic acid, or combinations of two or more of the above agents.

25 Preferred residues of chelating agents contain polycarboxylic acid groups and include: ethylenediamine-N, N, N',N'-tetraacetic acid (EDTA); N,N,N',N'',N''-

diethylene-triaminepentaacetic acid (DTPA); 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA); 1,4,7,10-tetraazacyclododecane-N,N',N''-triacetic acid (DO3A); 1-oxa-4,7,10-triazacyclododecane-N,N',N''-triacetic acid (OTTA); and trans(1,2)-cyclohexanodiethylenetriamine pentaacetic acid (CDTPA).

Preferred residues of chelating agents contain polycarboxylic acid groups and include: B4A, P4A, TMT, DCDTPA, PheMT, macroPheMT, and macroTMT;



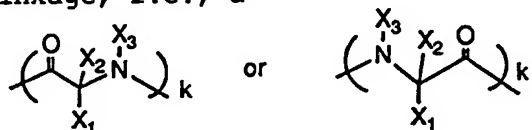
In one aspect, other suitable residues of chelating agents comprise proteins modified for the chelation of metals such as technetium and rhenium as described in U.S. Patent No. 5,078,985, the disclosure of which is hereby incorporated by reference.

In another aspect, suitable residues of chelating agents are derived from N_3S and N_2S_2 containing compounds, as for example, those disclosed in U.S. Patent Nos. 4,444,690; 4,670,545; 4,673,562; 4,897,255; 4,965,392; 4,980,147; 4,988,496; 5,021,556 and 5,075,099.

Other suitable residues of chelating agents are described in PCT/US91/08253, the disclosure of which is hereby incorporated by reference. If Q comprises the residue of multiple chelating agents, such agents can be linked together by a linking group such as described above.

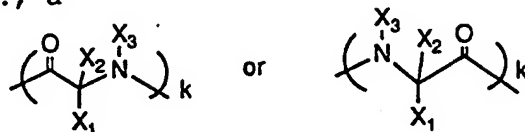
The residue of the chelating agents Q are independently linked to the other components of this invention through a chemical bond or a linking group. Preferred linking groups include nitrogen atoms in groups such as amino, imido, nitrilo and imino groups; alkylene, preferably containing from 1 to 18 carbon atoms such as methylene, ethylene, propylene, butylene and hexylene, such alkylene optionally being interrupted by 1 or more heteroatoms such as oxygen, nitrogen and sulfur or heteroatom-containing groups; carbonyl;

sulfonyl; sulfinyl; ether; thioether; ester, i.e.,
 carbonyloxy and oxycarbonyl; thioester, i.e.,
 carbonylthio, thiocarbonyl, thiocarbonyloxy, and
 oxythiocarboxy; amide, i.e., iminocarbonyl and
 5 carbonylimino; thioamide, i.e., iminothiocarbonyl and
 thiocarbonylimino; thio; dithio; phosphate; phosphonate;
 urelene; thiourelene; urethane, i.e.,
 iminocarbonyloxy, and oxycarbonylimino; thiourethane,
 i.e., iminothiocarbonyloxy and oxythiocarbonylimino; an
 10 amino acid linkage, i.e., a



group wherein $k=1$ and X_1 , X_2 , X_3 independently are H,
 alkyl, containing from 1 to 18, preferably 1 to 6 carbon
 atoms, such as methyl, ethyl and propyl, such alkyl
 15 optionally being interrupted by 1 or more heteroatoms
 such as oxygen, nitrogen and sulfur, substituted or
 unsubstituted aryl, containing from 6 to 18, preferably
 6 to 10 carbon atoms such as phenyl, hydroxyiodophenyl,
 hydroxyphenyl, fluorophenyl and naphthyl, aralkyl,
 20 preferably containing from 7 to 12 carbon atoms, such as
 benzyl, heterocyclyl, preferably containing from 5 to 7
 nuclear carbon and one or more heteroatoms such as S, N,
 P or O, examples of preferred heterocyclyl groups being
 pyridyl, quinolyl, imidazolyl and thienyl;
 25 heterocyclylalkyl, the heterocyclyl and alkyl portions

of which preferably are described above; or a peptide linkage, i.e., a



group wherein $k > 1$ and each of X_1 , X_2 , and X_3 is

5 independently represented by a group as described for X_1 , X_2 , and X_3 above. Two or more linking groups can be used, such as, for example, alkyleneimino and iminoalkylene. It is contemplated that other linking groups may be suitable for use herein, such as linking groups commonly used in protein heterobifunctional and homobifunctional conjugation and crosslinking chemistry as described for L_1 or L_2 above. Especially preferred linking groups include amino groups which when linked to the residue of a chelating agent via an isothiocyanate group on the chelating agent form thiourea groups.

15 The linking groups can contain various substituents which do not interfere with the coupling reaction between the chelating agent Q and the other components of this invention. The linking groups can also contain substituents which can otherwise interfere with such reaction, but which during the coupling reaction, are prevented from so doing with suitable protecting groups commonly known in the art and which substituents are regenerated after the coupling reaction by suitable deprotection.

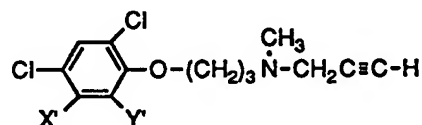
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25 The linking groups can also contain

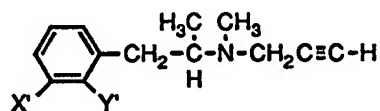
substituents that are introduced after the coupling reaction. For example, the linking group can be substituted with substituents such as halogen, such as F, Cl, Br or I; an ester group; an amide group; alkyl, preferably containing from 1 to about 18, more preferably, 1 to 4 carbon atoms such as methyl, ethyl, propyl, isopropyl, butyl, and the like; substituted or unsubstituted aryl, preferably containing from 6 to about 20, more preferably 6 to 10 carbon atoms such as phenyl, naphthyl, hydroxyphenyl, iodophenyl, hydroxyiodophenyl, fluorophenyl and methoxyphenyl; substituted or unsubstituted aralkyl, preferably containing from 7 to about 12 carbon atoms, such as benzyl and phenylethyl; alkoxy, the alkyl portion of which preferably contains from 1 to 18 carbon atoms as described for alkyl above; alkoxyaralkyl, such as ethoxybenzyl; substituted or unsubstituted heterocyclyl, preferably containing from 5 to 7 nuclear carbon and heteroatoms such as S, N, P or O, examples of preferred heterocyclyl groups being pyridyl, quinolyl, imidazolyl and thienyl; a carboxyl group; a carboxyalkyl group, the alkyl portion of which preferably contains from 1 to 8 carbon atoms; or the residue of a chelating group.

e) Residues of MAO active site binding derivatives,
MAO substrate analogs:

MAO substrate analogs are those which will fit the
active site of the enzyme and include, for example,
those with an aromatic ring and a side chain with an
amino group located in the ring's plane. The distance
between the center of the nitrogen and the ring should
be 0.5 to 0.55 nanometers. As non-limiting examples, in
systems A and B, specific examples of the MAO active
site binding ligands useful in this invention include
propargyl amine derivatives such as derivatives of
clorgyline, a suicide inhibitor of MAO-A, or of
deprenyl, an inhibitor of MAO-B, shown below:



Clorgyline



Deprenyl

In these structures the groups X' and Y',
preferably one of such groups, represents a possible
site for the attachment by a linking group L₂ to a
chelating group in the RDA of System A or by a linking
group L₁ to an immunoreactive group in the NRTIR or
System B. Preferably, one of the groups X' and Y' is
selected from the group consisting of H, a halogen such
as F, Cl, Br, and I, an alkyl group of 1 to 6 carbons, a

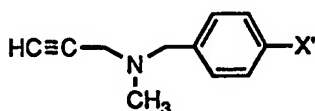
carboxylic acid group, a carboxylic amide group, and an alkyl ether group wherein the alkyl group is as defined above, and the other of the groups X' and Y' comprises a group selected from the group consisting of a suitably substituted linear alkylene group containing from 1 to 12 carbons, a branched alkylene group containing from 2 to 12 carbons, a cyclic alkylene group of from 3 to 12 carbons, an ether group linked to an alkylene group where alkylene is defined above, an alkylene group as defined above containing one to 6 ether groups the oxygens in which are separated by 2 to 6 carbons such as a polyethylene glycolyl group or a polypropylene glycolyl group or a polyethylene-co-propylene glycolyl group, a methyleneoxyethylene group, and the like, an amido group -CO-NX₃- or -NX₃-CO- wherein X₃ is H or an alkyl group as defined above, an alkylene amido or an amido alkylene group wherein the alkylene and amido groups are as defined above, or peptidyl moiety as defined above to which residues of a chelator molecule can be attached (i.e., linking groups). Further substitution may also be possible on the groups adjacent to the amino groups as long as they fall within the description outlined above. The alkyl and alkylene groups may also contain one or more sites of unsaturation such as -CH=CH- and ethynyl groups.

Preferred analogs include those modified in such a way so as to permit or facilitate binding of one portion

of the ligand to the immunoreactive species in system B by means of a linking group L₁ and to the chelating species in system A by means of a linking group L₂.

Other related MAO inhibitory compounds which could be similarly derivatized so as to act as MAO substrate analogs include N-cyclopropyl-N-arylalkyl amines and drugs of the type exemplified by 3-[4-(3-cyanophenylmethoxy)phenyl]-5-(methoxymethyl)-2-oxazolidinone.

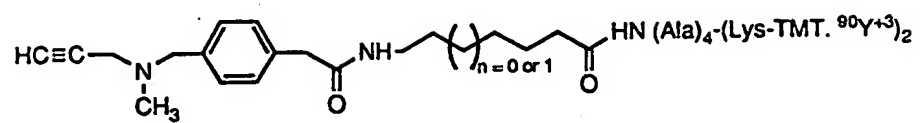
It is specifically contemplated that the MAO substrate analogs contemplated herein will form essentially irreversible attachments, such as via covalent bonds, with the elements of the active site of MAO. An especially preferred class of compounds that will form irreversible, covalent attachments with the elements of the active site of MAO are derivatives of pargyline.



Pargyline

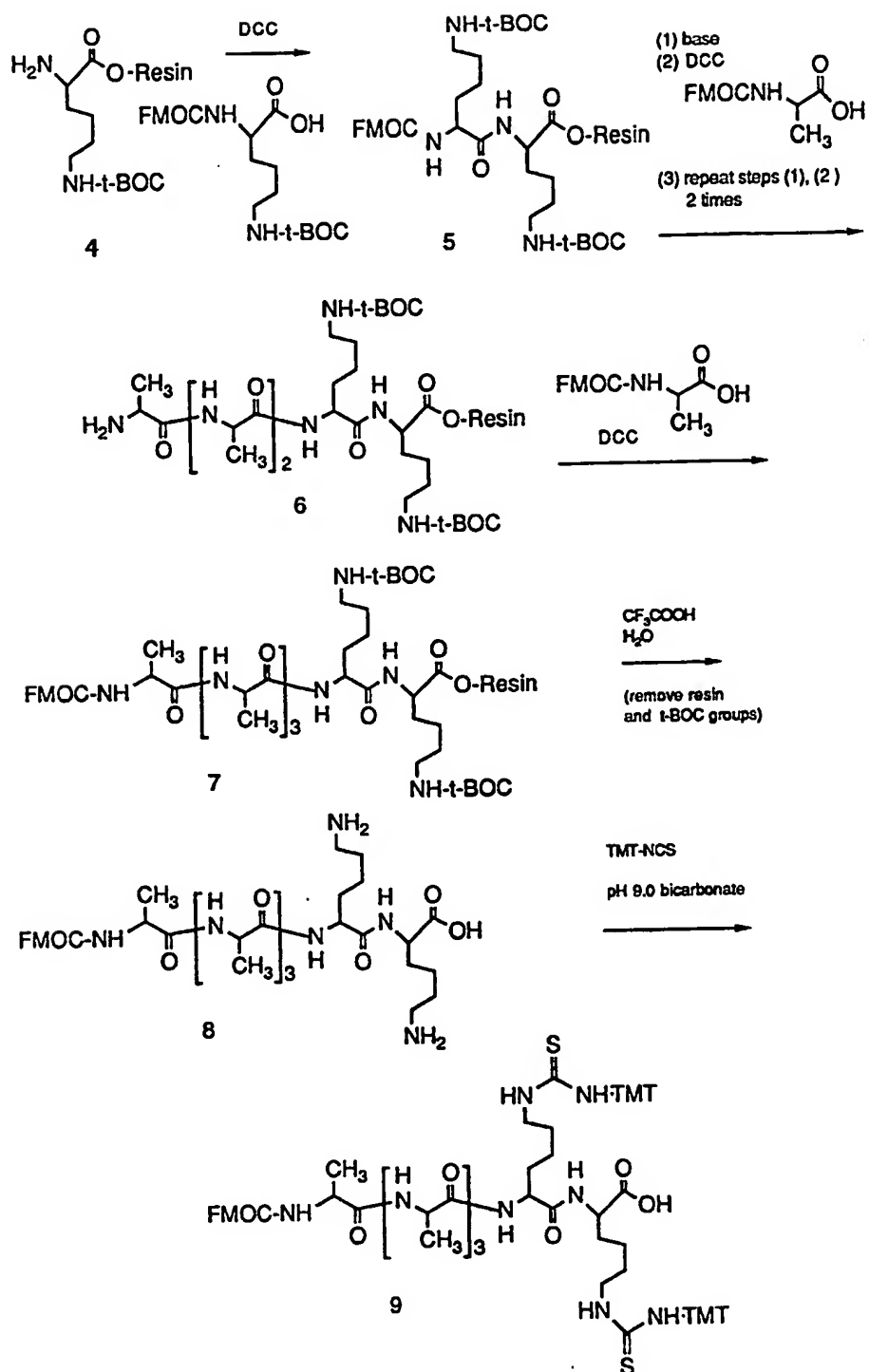
In this structure, the group X' is as defined above and represents a site for the attachment by a linking group L₂ to a chelating group in the RDA of System A or by a linking group L₁ to an immunoreactive group in the NRTIR or System B. Further substitution may also be possible on the groups adjacent to the amino group as long as the derivative falls within the description outlined above.

The preparation of a preferred RDA in system A,

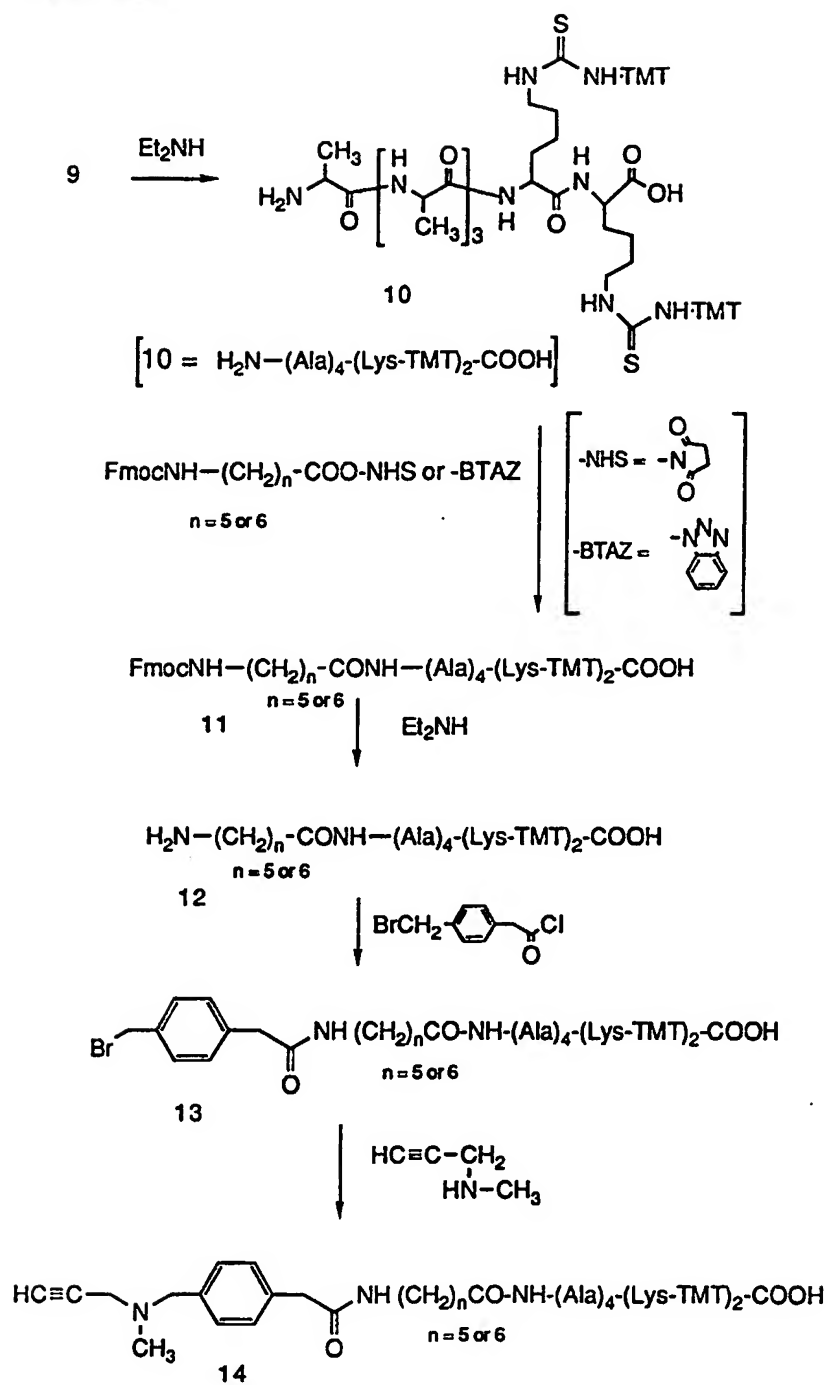


is outlined in Schemes 2, 3, and 4.

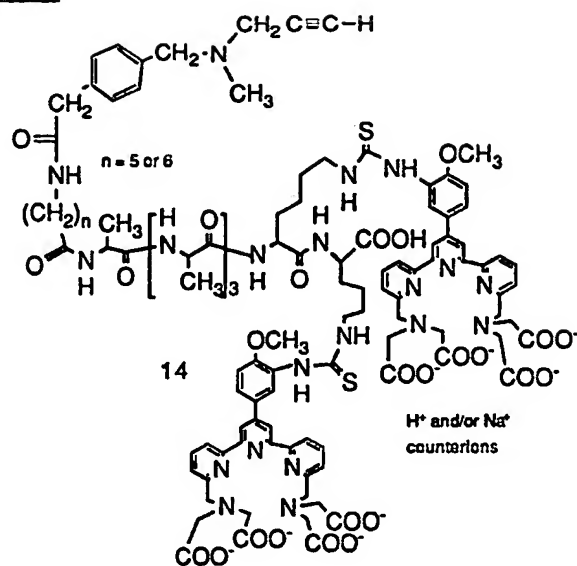
SCHEME 2



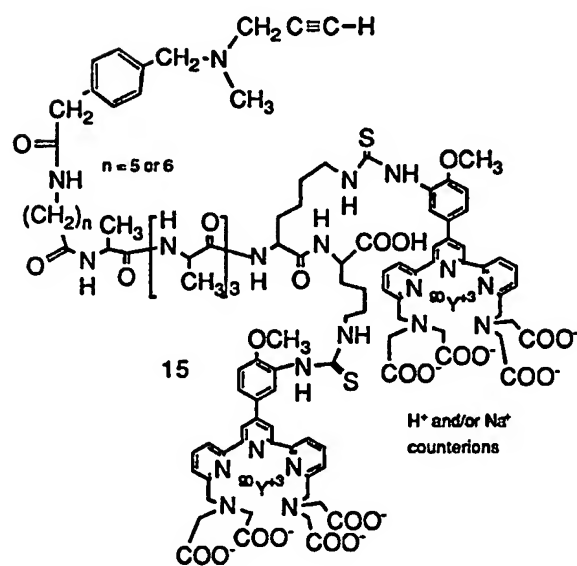
SCHEME 3



SCHEME 4



Radionuclide ion
(e.g., $^{90}Y^{3+}$, $^{32}P^{3-}$
in NaOAc buffer)



In the RDA of System A, preferred non-limiting examples of linking groups between the pargyline aromatic ring and a chelating group (e.g. TMT) comprise the residue of a 6-methylenecarbonylaminoheptanoic acid amide with the N-terminal amine of a peptide such as H₂N-(Ala)₄-Lys-Lys-OH (SEQ ID NO:1) as well as the residue of a 7-methylenecarbonylaminoheptanoic acid amide with the N-terminal amine of a peptide such as H₂N-(Ala)₄-Lys-Lys-OH (SEQ ID NO:1). In the peptide moiety of the linking group, the number of alanine (Ala) residues is preferably in the range of from 0 to 12, and the number of lysine (Lys) residues is preferably in the range from 2 to 20. In addition, the lysines can be contiguous or can be separated by spacer groups such as the residue of amino acids such as 6-aminohexanoic acid, 7-aminoheptanoic acid, alanine, glycine, valine, glutamic acid, aspartic acid, phenylalanine, serine, threonine, leucine, isoleucine, and other amino acids that will not interfere with the binding of the pargyline to the MAO active site or with the binding of the lysine amines to the chelator. Preferably, when the lysines are separated from each other with a spacing group, the spacing group between the lysines is selected from the group consisting of one or two 6-aminohexanoic acid residues, one or two 7-aminoheptanoic acid residues, 1 to 12 alanine residues, and 1 to 12 glycine residues.

In SCHEME 2, the t-butoxycarbonyl (t-Boc) epsilon amine blocked Lys-Lys (structure 5) is prepared using a dehydrative coupling method, for example, using DCC (dicyclohexylcarbodiimide) and two epsilon amine
5 protected lysine groups wherein one lysine (structure 4) is bound to a resin by an ester bond and has an unblocked alpha amino group, and wherein the other lysine has an unblocked carboxylic acid group and an FMOC-blocked alpha amino group. Alternatively, a
10 reagent such as benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) can be employed to react with a carboxylic acid to form a benzotriazoyloxy (BTAZ) ester which will react with the unblocked, N-terminal amine of an amino acid
15 sequence to form a peptide bond. The FMOC (9-fluorenylmethoxycarbonyl) group of resin linked Lys-Lys is then removed via base treatment, and 4 units of alanine are introduced as shown in SCHEME 2 to afford the resin bound bis(t-BOC) FMOC-blocked Ala-Ala-Ala-Ala-Lys-Lys-COO-resin (SEQ ID NO:2; structure 7). The
20 acidic removal of the t-BOC groups and acidic removal of the peptide from the resin affords the FMOC-blocked peptide, FMOC-NH-(Ala)₄-(Lys)₂-COOH (SEQ ID NO:3; structure 8). This material is then treated with TMT-
25 NCS in bicarbonate buffer at pH 9 to afford the bis-TMT derivatized FMOC-blocked peptide (structure 9). The FMOC group is then removed from the peptide as shown in

SCHEME 3 by treatment with diethylamine to afford the peptide $\text{H}_2\text{N}-(\text{Ala})_4(\text{Lys-TMT})_2-\text{COOH}$ (SEQ ID NO:4; structure 10). This peptide is then coupled to 6-(Fmoc-amino)-hexanoic acid or to 7-(Fmoc-amino)-heptanoic acid by means of an activated ester such as an N-hydroxysuccinimide (NHS) ester which is formed by the reaction of N-hydroxysuccinimide with the adduct of dicyclohexylcarbodiimide and 6-(Fmoc-amino)-hexanoic acid or which is formed by the reaction of N-hydroxysuccinimide with the adduct of dicyclohexylcarbodiimide and 7-(Fmoc-amino)-heptanoic acid, respectively, or by means of an activated ester such as a benzotriazol-1-yl ester formed by the reaction of benzotriazol-1-yl- with tris(dimethylamino)phosphonium hexafluorophosphate (BOP) with 6-Fmoc-aminohexanoic acid or with 7-Fmoc-aminoheptanoic acid, respectively. The resulting Fmoc-protected aminomethylene amide linked peptide (structure 11, $n = 5$ or 6 , respectively, for hexanoic and heptanoic spacing groups) is then treated with diethylamine to remove the Fmoc blocking group to produce the free amine derivative (structure 12, $n = 5$ or 6). This material is then acylated in the presence of sodium carbonate at ice temperature with 4-(bromomethyl)phenylacetyl chloride (cf. R. R. Rando, *Molecular Pharmacology*, 1977, 13, 726-734) to form the amide (structure 13, $n = 5$ or 6) which is then treated with N-methylpropargylamine to afford

the pargyline derivative (structure 14, $n = 5$ or 6).

This material can be purified and isolated using reverse phase and size exclusion high pressure liquid

chromatography followed by removal of solvent, for

5 example, by lyophilization. The desired RDA can be

obtained as shown in SCHEME 4 by treatment of the

pargyline derivative (structure 14, $n = 5$ or 6) with a

radioactive metal ion. When $^{90}\text{Y}^{+3}$ is used, a solution

containing a desired number of mCi of $^{90}\text{Y}^{+3}$ (as $^{90}\text{YCl}_3$ in

10 hydrochloric acid) is preferably added at room

temperature to a solution of the pargyline derivative in

(phosphate free) 0.5 M sodium acetate buffer at about pH

6.0 in deionized water.

Employing the similar synthetic methods, but using

15 a blocked Gly derivative instead of a blocked Ala

derivative, the analogous Gly-containing pargyline

derived materials are obtained.

Employing the similar synthetic methods, but using

a blocked Glu derivative, the analogous Glu-containing

20 pargyline derived materials are obtained.

Enantiomerically pure L- and pure D-amino acid

derivatives, as well as racemic mixtures of D- and L-

amino acid enantiomers of the above described pargyline

derivatives are also useful in this invention.

25 Additional chelating agents and radionuclides bound

to chelating agents are incorporated by preparing, for

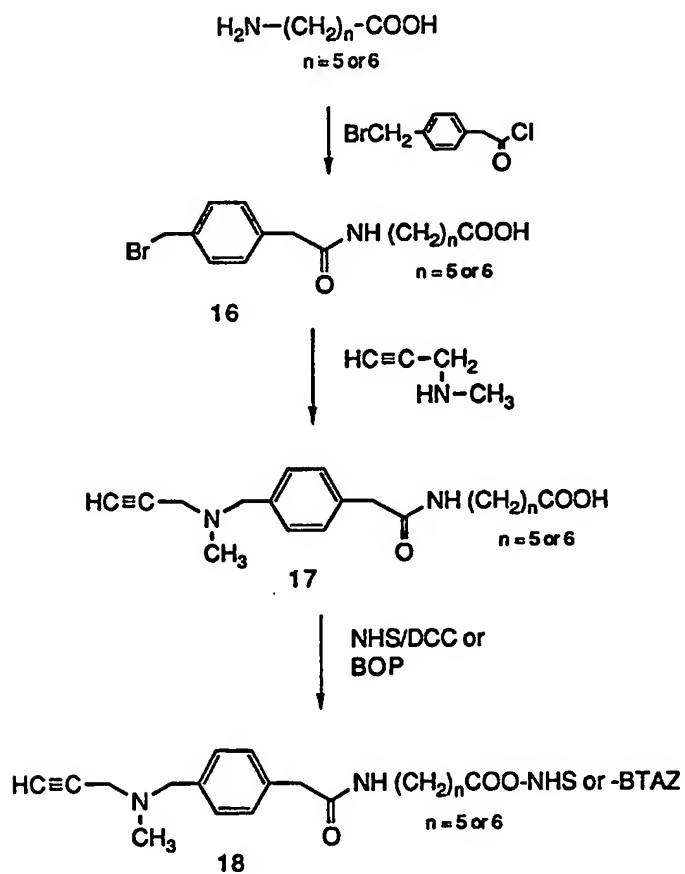
example, analogous peptides comprised of additional Lys-

TMT and Lys-TMT-radionuclide groups. Preferably, the number of such Lys-TMT and Lys-TMT-radionuclide residues is from 1 to about 20, and more preferably from 2 to about 10.

5 In the System B, the NRTIR is comprised of one or more ligands that have an affinity for binding to a MAO active site each with a suitably substituted linking group (L_1) conjugated to the immunoreactive group (Z). Preferably, said ligand that has an affinity for binding
10 to a MAO active site is comprised of a 4-substituted pargyline residue linked to Z by a linking group (L_1) as defined above. The NRTIR preferably contains 2 to about 10 of such groups, more preferably 2 to about 4.

The synthesis of a preferred class of pargyline
15 derivatives that is useful in the NRTIR of System B is outlined in SCHEME 5. Thus, 6-aminohexanoic acid or 7-aminoheptanoic acid are acylated with 4-(bromomethyl)phenylacetyl chloride to afford the corresponding amide derivative (structure 16, $n = 5$ or
20 6, respectively). The amide is then treated with N-methylpropargylamine to afford the pargyline derivative (structure 17, $n = 5$ or 6). This material can be purified and isolated using high pressure liquid chromatography followed by removal of solvent, for
25 example, by lyophilization. This pargyline derivative is then converted to an activated ester for coupling to a protein by reaction of the carboxylic acid group with

5 NHS and DCC or with BOP as described above. The active ester (structure 18, $n = 5$ or 6) can then be reacted with, for example, a lysine amine group on an immunoreactive group Z to afford a covalent bond between the immunoreactive group and linking group (L_1) which is attached to the pargyline moiety. The NRTIR of System B comprises from one to about 20 of such pargyline containing groups, preferably from one to about 6.

SCHEME 5

10

f) Radionuclide - Chelation of the Metals

In one embodiment, both in system A and system B, it is desirable that the metal ion can be easily complexed to the chelating agent, for example, by merely exposing or mixing an aqueous solution of the chelating agent-containing moiety with a metal salt in an aqueous solution preferably having a pH in the range of about 4 to about 11. The salt can be any water soluble salt of the metal such as a halogen salt, but preferably such salts are selected so as not to interfere with the binding of the metal ion with the chelating agent. The chelating agent-containing moiety is preferably in aqueous solution at a pH of between about 5 and about 9, more preferably between pH about 6 to about 8. The chelating agent-containing moiety optionally is mixed with buffers such as citrate, acetate, phosphate and borate to produce the optimum pH. Preferably, the buffer salts are selected so as not to interfere with the subsequent binding of the metal ion to the chelating agent.

The radioactive chelating agent-containing moiety of this invention can contain any ratio of metal radionuclide ion to chelating agent that is effective in therapeutic and diagnostic imaging applications. In therapeutic applications, in preferred embodiments, the mole ratio of metal ion per chelating agent is from about 1:100 to about 1:1. In diagnostic imaging

applications, in preferred embodiments, the mole ratio of metal ion per chelating agent is from about 1:1,000 to about 1:1.

5 Non-radioactive metals:

In another embodiment, the metal ion of this invention can comprise a non radioisotope. The metal ions can be selected from, but are not limited to, elements of groups IIA through VIA. Preferred metals
10 include those of atomic number 12, 13, 20, the transition elements 21 - 33, 38 - 52, 56, 72 -84 and 88 and those of the lanthanide series (atomic number 57 - 71).

15 Radioactive metals:

In another embodiment, the metal ion of this invention can comprise a radionuclide. The radionuclide can be selected, for example, from radioisotopes of Sc, Fe, Pb, Ga, Y, Bi, Mn, Cu, Cr, Zn, Ge, Mo, Tc, Ru, In,
20 Sn, Sr, Sm, Lu, Sb, W, Re, Po, Ta and Tl. Preferred radionuclides include ^{44}Sc , ^{64}Cu , ^{67}Cu , ^{111}In , ^{212}Pb , ^{68}Ga , ^{90}Y , ^{153}Sm , ^{212}Bi , $^{99\text{m}}\text{Tc}$, ^{186}Re and ^{188}Re . Of these, especially preferred is ^{90}Y . These metals can be atomic or preferably ionic.

25

Fluorescent metals:

In another embodiment, the metal ion of this invention can comprise a fluorescent metal ion. The fluorescent metal ion can be selected from, but is not limited to, metals of atomic number 57 to 71. Ions of the following metals are preferred: La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu. Eu is especially preferred.

Paramagnetic metals:

In another embodiment, the metal ion of this invention can comprise one or more paramagnetic elements which are suitable for the use in MRI applications. The paramagnetic element can be selected from elements of atomic number 21 to 29, 43, 44 and 57 to 71. The following elements are preferred: Cr, V, Mn, Fe, Co, Ni, Cu, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu. Mn, Gd, and Dy are especially preferred.

Other embodiments:

In another embodiment of this invention, the simultaneous use of two or more metal ions in combination with one another in the same chelating agent containing moiety is specifically contemplated. For example, the use of a therapeutically effective dose of a radionuclide such as $^{90}\text{Y}^{+3}$ together in the same chelating agent containing moiety sample with a

diagnostic imaging effective dose of a paramagnetic ion such as Gd^{+3} , the molar concentration of the latter ion being typically in excess with respect to that of the former ion in the conjugated complex, would permit the simultaneous magnetic resonance imaging of at least a portion of the tissue of a host patient during therapeutic treatment of said patient.

In another embodiment of this invention, the use of radioisotopes of iodine is specifically contemplated.

For example, if the RDA of System A or of System B comprises substituents that can be chemically substituted by iodine in a covalent bond forming reaction to iodine, such as for example, substituents containing hydroxyphenyl functionality, such substituents can be labeled by methods well known in the art with a radioisotope of iodine. The thus covalently linked iodine species can be used in the aforementioned fashion in therapeutic and diagnostic imaging applications.

Advantages:

There are many advantages of the present invention.

These include the following:

therapeutically effective doses of radioisotope can be delivered to a diseased tissue site;

the delivery of radioisotope is site specific;

delivery of radionuclide to a diseased tissue site can be achieved in amplification over that which can be achieved with a single stage delivery system;

5 the system will reduce the exposure of non-target tissues to damage from radiation;

the binding of the ligand to the receptor is essentially irreversible and selective;

the system can be used in both therapeutic and diagnostic imaging applications;

10 the above-described NRTIR can accumulate at a tumor site in vivo while it is not accumulated at normal tissue sites;

the in vivo residence half life of the above-described NRTIR is long enough to permit its
15 accumulation at a tumor site;

the in vivo residence half life of the above-described RDA is shorter than the residence half life of the above-described NRTIR;

20 the portion of the above described RDA that does not bind to tumor associated NRTIR is rapidly cleared from the patient;

with respect to the same degree of modification of a targeting immune reagent by a directly labeled radionuclide or a chelate containing a radionuclide, an
25 amplification of the number of radionuclides per site of modification per targeting immune reagent can be obtained;

the above-described NRTIR can comprise a wide variety of immune reactive groups, linking groups, and MAO active site residues in system A or MAO active site binding ligand residues in system B;

5 the above-described RDA can comprise a wide variety of spacing, linking and chelating groups, radionuclides, and MAO active site binding ligand residues in system A or MAO active site residues in system B;

10 compounds are provided having a specificity to accumulation in tumors; and

a wide variety of specified compositions, size and molecular weight can be prepared in accordance with this invention.

15 Other advantageous features of this invention will become readily apparent upon reference to the following description of the preferred embodiments.

Effective methods of administration and dosages:

20 In a preferred embodiment, an effective dose of an RDA of System A or System B as described above in a pharmaceutically acceptable medium is prepared by exposing a composition of a precursor of an RDA (said precursor comprising a residue of a ligand that has an ability to covalently bind to a MAO active site, a
25 linking group, and a residue of a chelating agent in System A and of a residue of a MAO active site, a linking group, and a residue of a chelating agent in

System B) to a composition containing a radioactive metal ion such that the molar amount of said radionuclide metal ion is less than the molar amount of the chelating groups comprising the RDA, said duration of exposure lasting an effective time to permit uptake of said metal ion into said RDA.

In a preferred embodiment, an effective dose of a NRTIR of System A or System B as described above in a pharmaceutically acceptable medium is administered to a patient and said NRTIR is allowed to accumulate at the target site such as at a tumor site in said patient. Subsequently, at an effective time, an effective dose of a RDA as described above in a pharmaceutically acceptable medium is administered to said patient, and said RDA is allowed to accumulate at the target site, said target site being the said NRTIR accumulated at said tumor site in said patient.

The present invention includes one or more NRTIR as described above and one or more RDA as described above formulated into compositions together with one or more non-toxic physiologically acceptable carriers, adjuvants or vehicles which are collectively referred to herein as carriers, for parenteral injection for oral administration in solid or liquid form, for rectal or topical administration, or the like.

The compositions can be administered to humans and animals either orally, rectally, parenterally

(intravenous, by intramuscularly or subcutaneously), intracisternally, intravaginally, intraperitoneally, intravesically, locally (powders, ointments or drops), or as a buccal or nasal spray.

5 Compositions suitable for parenteral injection may comprise physiologically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions and sterile powders for reconstitution into sterile injectable solutions or dispersions. Examples of
10 suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (propyleneglycol, polyethyleneglycol, glycerol, and the like), suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as
15 ethyl oleate. Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants.

20 These compositions may also contain adjuvants such as preserving, wetting, emulsifying, and dispensing agents. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to
25 include isotonic agents, for example sugars, sodium chloride and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by

the use of agents delaying absorption, for example, aluminum monostearate and gelatin.

5 Solid dosage forms for oral administration include capsules, tablets, pills, powders and granules. In such solid dosage forms, the active compound is admixed with at least one inert customary excipient (or carrier) such as sodium citrate or dicalcium phosphate or (a) fillers or extenders, as for example, starches, lactose, sucrose, glucose, mannitol and silicic acid, (b) 10 binders, as for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose and acacia, (c) humectants, as for example, glycerol, (d) disintegrating agents, as for example, agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain complex silicates and sodium carbonate, 15 (e) solution retarders, as for example paraffin, (f) absorption accelerators, as for example, quaternary ammonium compounds, (g) wetting agents, as for example, cetyl alcohol and glycerol monostearate, (h) adsorbents, 20 as for example, kaolin and bentonite, and (i) lubricants, as for example, talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate or mixtures thereof. In the case of capsules, tablets and pills, the dosage forms may also 25 comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin

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formulations, eye ointments, powders and solutions are also contemplated as being within the scope of this invention.

5 Actual dosage levels of active ingredients in the compositions of the present invention may be varied so as to obtain an amount of active ingredient that is effective to obtain a desired therapeutic response for a particular composition and method of administration. The selected dosage level therefore depends upon the
10 desired therapeutic effect, on the route of administration, on the desired duration of treatment and other factors.

The total daily dose of the compounds of this invention administered to a host in single or divided
15 dose may be in amounts, for example, of from about 1 nanomol to about 5 micromols per kilogram of body weight. Dosage unit compositions may contain such amounts of such submultiples thereof as may be used to make up the daily dose. It will be understood, however,
20 that the specific dose level for any particular patient will depend upon a variety of factors including the body weight, general health, sex, diet, time and route of administration, rates of absorption and excretion, combination with other drugs and the severity of the
25 particular disease being treated.

In another embodiment, the present invention is directed to a method of diagnosis and comprises the

administration of a contrast effective amount of the compositions of the present invention to a mammal in need of such diagnosis. A method for diagnostic imaging for use in medical procedures in accordance with this invention comprises administering to the body of a test subject in need of a diagnostic image an effective contrast producing amount of the above-described NRTIR containing composition. The NRTIR is allowed to bind to sites on cells of a tissue of interest, the unbound NRTIR is cleared from the environs of the tissue and an RDA containing composition as described above in a pharmaceutically acceptable medium is administered to the subject. The radioactive targeting reagent is allowed to accumulate at the target site, said target site being the non-radioactive targeting immunoreagent accumulated at the sites on cells of a tissue of interest in said subject. The image pattern can then be visualized, for example, by radiosciintigraphy, by a radiation sensitive detector and signal amplifier.

In addition to human patients, the test subject can include mammalian species such as rabbits, dogs, cats, monkeys, sheep, pigs, horses, bovine animals and the like.

Alternatively, the NRTIR may be reacted with a diagnostic imaging effective amount of a reagent comprised of a radionuclide prior to administration to the environs of a tissue of interest of a patient

undergoing such diagnostic imaging, waiting for an effective period of time during which time said NRTIR will bind to sites on cells of said tissue of interest and during which time unbound NRTIR will be removed from the environs of said tissue and then obtaining an image as a function of time of all or part of said tissue of interest. When the image of all or part of said tissue of interest is optimal, a diagnostic imaging or a therapeutically effective amount of RDA containing the same or a different radionuclide as that employed on the NRTIR is administered to said tissue of interest of said patient.

Alternatively, at least a portion of the body containing the administered contrast agent is exposed to x-rays or to a magnetic field to produce an x-ray or magnetic resonance image pattern corresponding to the presence of heavy elements such as iodine and heavy metal ions in the contrast agent. The image pattern can then be visualized.

In x-ray imaging, transmitted radiation is used to produce a radiograph based upon overall tissue attenuation characteristics. X-rays pass through various tissues and are attenuated by scattering, i.e., reflection or refraction or energy absorption. However, certain body organs, vessels and anatomical sites exhibit so little absorption of x-ray radiation that radiographs of these body portions are difficult to

obtain. To overcome this problem, radiologists routinely introduce an x-ray absorbing medium containing a contrast agent into such body organs, vessels and anatomical sites.

5 Any x-ray visualization technique, preferably, a high contrast technique such as computed tomography, can be applied in a conventional manner. Alternatively, the image pattern can be observed directly on an x-ray sensitive phosphor screen-silver halide photographic
10 film combination.

 Visualization with a magnetic resonance imaging system when paramagnetic ions are used can be accomplished with commercially available magnetic imaging systems such as a General Electric 1.5 T Signa
15 imaging system [1H resonant frequency 63.9 megahertz (MHz)]. Commercially available magnetic resonance imaging systems are typically characterized by the magnetic field strength used, with a field strength of 2.0 Tesla as the current maximum and 0.2 Tesla as the
20 current minimum.

 For a given field strength, each detected nucleus has a characteristic frequency. For example, at a field strength of 1.0 Tesla, the resonance frequency for hydrogen is 42.57 MHz; for phosphorus-31 it is 17.24
25 MHz; and for sodium-23 it is 11.26 MHz.

 A contrast effective amount of the compositions of the present invention is that amount necessary to

provide tissue visualization with, for example, magnetic resonance imaging or x-ray imaging. Means for determining a contrast effective amount in a particular subject will depend, as is well known in the art, on the nature of the magnetically reactive material used, the mass of the subject being imaged, the sensitivity of the magnetic resonance or x-ray imaging system and the like.

After administration of the compositions of the present invention, the subject mammal is maintained for a time period sufficient for the administered compositions to be distributed throughout the subject and enter the tissues of the mammal. Typically, a sufficient time period is from about 20 minutes to about 2 weeks or more and, preferably from about 20 minutes to about 1 week.

The following examples further illustrate the invention and are not to be construed as limiting of the specification and claims in any way. Specific embodiments of the invention are illustrated in the following examples.

EXAMPLES

The following examples relate to System A, i.e.,

SYSTEM A

	Non-Radioactive Targeting ImmunoReagent NRTIR	Radioactive Delivery Agent RDA
1	Immunoreactive group + (linking group + receptor) _n	Ligand + (chelating agent + radionuclide) _m
2	Z-(L ₁ -Rec) _n	D-(L ₂ -Q-M) _m
3	Antibody-(L ₁ -MAO) _n	Pargyline-(L ₂ -TMT- ⁹⁰ Y) _m

wherein:

5 Z is the residue of an immunoreactive group, preferably an antibody;

Rec is the residue of a receptor, preferably a monoamine oxidase (MAO);

10 D is the residue of a ligand that has an affinity for covalent binding to the receptor, and preferably said ligand is pargyline, an inhibitor of monoamine oxidase;

L₁ and L₂ are each independently the residue of a linking group that may contain a spacing group;

15 Q is the residue of a chelating group such as TMT, above;

M is a radionuclide, preferably ⁹⁰Y; and

n and m are each independently an integer greater than zero.

5 Example 1

Synthesis of Fmoc-HN-Ala-Ala-Ala-Ala-Lys-Lys-OH (SEQ ID NO:3)

10 The linear N-alpha-Fmoc-protected peptide, Fmoc-HN-Ala-Ala-Ala-Ala-Lys-Lys-OH (SEQ ID NO:3), is synthesized via solid-phase methodology on an ABI 430A Automated Peptide Synthesizer. The solid support used in the synthesis is a 4-alkoxybenzyl alcohol polystyrene resin
15 (Wang resin). The N-alpha-Fmoc protecting group is used throughout the synthesis; the amines on the side chain of Lys are protected with t-BOC. The peptide chain is assembled using the ABI FastMoc™ software protocols for Fmoc-chemistry (0.25 mmole scale, HBTU activated
20 couplings, 4 fold excess of amino acid, 1 hour). The peptide is removed from the resin and t-BOC groups are removed from the lysine amines by treatment of the peptide-resin with 15 mL of a 95:5 solution of trifluoroacetic acid in water in a sealed vessel
25 followed by shaking at room temperature for 2 hours. The mixture is then filtered using a scintered glass funnel. The filtrate volume is then reduced to about 3 mL by rotoevaporation, and the peptide is precipitated

by dropping the oil into a centrifuge tube containing 50 mL of ether. The peptide is separated by centrifugation, the ether is decanted, the solid is washed with more ether and then allowed to air dry.

5

Example 2

Synthesis of Fmoc-HN-Ala-Ala-Ala-Ala-(Lys-TMT)-(Lys-TMT)-OH (SEQ ID NO:5)

10

The N-alpha-Fmoc-protected peptide, Fmoc-HN-Ala-Ala-Ala-Ala-Lys-Lys-OH (SEQ ID NO:3), from Example 1 (20 mM) is dissolved in a saturated sodium bicarbonate solution (pH 9) in deionized water (50 mL) containing

15

dimethylsulfoxide (DMSO, 5 mL). This solution is treated with TMT-NCS (25 mM), and the reaction is allowed to continue for 12 hours at room temperature.

This solution is then treated with more TMT-NCS (25 mM), and the reaction is allowed to continue for 24 hours at

20

room temperature. The reaction is followed by size exclusion HPLC until all the TMT-NCS and all of the peptide are reacted. The desired lysyl-TMT-containing peptide is isolated by chromatography on a Shodex WS-803F size exclusion column using a UV-visible detector monitoring the absorption of TMT. A solid sample can be isolated from solution by lyophilization.

25

Example 3

Synthesis of $\text{H}_2\text{N-Ala-Ala-Ala-Ala-(Lys-TMT)-(Lys-TMT)-OH}$
(SEQ ID NO:4)

5

The N-alpha-Fmoc-protected peptide, Fmoc-HN-Ala-Ala-Ala-Ala-(Lys-TMT)-(Lys-TMT)-OH (SEQ ID NO:5), from Example 2 (10 mM) is dissolved in 50 mL of deionized water and 50 mL of dimethylsulfoxide, and then treated with 25 mM of N,N-diethylamine. The reaction vessel is sealed and warmed to 40 °C for 12 hours. The reaction is cooled, and the desired product is isolated by HPLC using a Shodex WS-803F size exclusion column and a UV-visible detector monitoring the absorption of TMT. A solid sample is isolated from solution by lyophilization.

15

Example 4

Synthesis of Fmoc-HN-(CH₂)₅-COOH

20

A solution of 6-aminohexanoic acid (100 mM, Aldrich) in chloroform containing diisopropylethylamine (200 mM) is treated with 9-fluorenylmethyl chloroformate (100 mM, Aldrich) at room temperature and allowed to stir overnight. The reaction mixture is washed twice with cold 0.1 N HCl solution, once with water, once with saturated sodium chloride solution, and dried over

25

anhydrous sodium sulfate. The desired product is isolated by filtration and evaporation of the solvent.

Example 5

5

Synthesis of Fmoc-HN-(CH₂)₅-COO-BTAZ

A solution of Fmoc-HN-(CH₂)₅-COOH (10 mM) from Example 4 in chloroform is treated with benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP, 10 mM) to produce the desired activated ester. A crude product is isolated by evaporation of the solvent on a rotary evaporator.

15

Example 6

Synthesis of Fmoc-HN-(CH₂)₅-CO-HN-Ala-Ala-Ala-Ala-(Lys-TMT)-(Lys-TMT)-OHM (SEQ ID NO:6)

20

The crude reaction product from Example 5 is treated with 10 mM of H₂N-Ala-Ala-Ala-Ala-(Lys-TMT)-(Lys-TMT)-OH (SEQ ID NO:4) from Example 3 in 50 mL of deionized water and 50 mL of dimethylsulfoxide containing 1 mL of pyridine. The reaction is allowed to proceed overnight at room temperature.

25

Example 7

Synthesis of $\text{H}_2\text{N}-(\text{CH}_2)_5-\text{CO}-\text{HN}-\text{Ala}-\text{Ala}-\text{Ala}-\text{Ala}-(\text{Lys}-\text{TMT})-(\text{Lys}-\text{TMT})-\text{OH}$ (SEQ ID NO:7) (formation of $(\text{L}_2-\text{Q})_m$)

5

The crude reaction product from Example 6 is treated with diethylamine according to the procedure of Example 3. The desired product is purified by HPLC using a Shodex WS-803F size exclusion column and a UV-visible
10 detector monitoring the absorption of TMT. A solid sample is isolated from solution by lyophilization.

Example 8

15 Synthesis of $\text{Br}-\text{CH}_2-\text{C}_6\text{H}_5-\text{CH}_2-\text{CO}-\text{HN}-(\text{CH}_2)_5-\text{CO}-\text{HN}-(\text{Ala})_4-(\text{Lys}-\text{TMT})_2-\text{OH}$ (SEQ ID NO:8)

A solution of $\text{H}_2\text{N}-(\text{CH}_2)_5-\text{CO}-\text{HN}-\text{Ala}-\text{Ala}-\text{Ala}-\text{Ala}-(\text{Lys}-\text{TMT})-(\text{Lys}-\text{TMT})-\text{OH}$ (SEQ ID NO:7; 1 mM) from Example 7 in 50 mL
20 of deionized water and 50 mL of DMSO is saturated with sodium carbonate in an ice bath. The peptide is treated with a solution of 4-bromomethylphenylacetyl chloride (2 mM) in 20 mL of tetrahydrofuran. The reaction is
25 stirred at ice temperature for 30 min and used immediately.

Example 9

Synthesis of H-CC-CH₂-N(CH₃)-CH₂-C₆H₆-CH₂-CO-HN-(CH₂)₅-
CO-HN-(Ala)₄-(Lys-TMT)₂-OH (SEQ ID NO:9) (formation of
D-(L₂-Q)_m)

To the crude reaction product of Example 8 is added 10
mL of N-methylpropargylamine (Aldrich), and the reaction
mixture is stirred for 6 hours at room temperature. The
desired product is isolated by HPLC using a Shodex WS-
803F size exclusion column and a UV-visible detector
monitoring the absorption of TMT. A solid sample is
isolated from solution by lyophilization.

Example 10

Radionuclide, ⁹⁰Y³⁺, chelated to H-CC-CH₂-N(CH₃)-CH₂-
C₆H₆-CH₂-CO-HN-(CH₂)₅-CO-HN-(Ala)₄-(Lys-TMT)₂-OH (SEQ ID
NO:9) (formation of D-(L₂-Q-M)_m)

A volume of radioactive ⁹⁰YCl₃ (⁹⁰Y in 0.04 M
hydrochloric acid at a specific activity of >500 Ci/mg;
Amersham-Medipysics) is neutralized using two volumes
of 0.5 M sodium acetate pH 6.0 and added to a solution
of H-CC-CH₂-N(CH₃)-CH₂-C₆H₆-CH₂-CO-HN-(CH₂)₅-CO-HN-
(Ala)₄-(Lys-TMT)₂-OH (SEQ ID NO:9) from Example 9 in
deionized water buffered with 0.5 M sodium acetate at pH

6.0 at room temperature. The molar ratio of TMT to ^{90}Y is greater than one at all times. The chelation of the ^{90}Y is allowed to proceed for one hour. The labeling efficiency is determined by removing 1.0 mL of the sample and spotting it on to a Gelman ITLC-SG strip. The strip is developed in a glass beaker containing 0.1 M sodium citrate, pH 6.0 for a few minutes until the solvent front has reached three-quarters of the way to the top of the paper. The strip is inserted into a System 200 Imaging Scanner (Bioscan) which is optimized for ^{90}Y and which is controlled by a Compaq 386/20e computer. In this system free ^{90}Y migrates at the solvent front while the peptide containing the TMT chelated to ^{90}Y remains near the origin. In excess of 97% of the added ^{90}Y is taken up by the $\text{H-CC-CH}_2\text{-N(CH}_3\text{)-CH}_2\text{-C}_6\text{H}_6\text{-CH}_2\text{-CO-HN-(CH}_2\text{)}_5\text{-CO-HN-(Ala)}_4\text{-(Lys-TMT)}_2\text{-OH}$ (SEQ ID NO:9) to form the desired ^{90}Y -chelated product.

The following examples illustrate the construction of a conjugate between monoamine oxidase and an antibody. ING-1 (a chimeric IgG₁ antibody) is a non-limiting example of such an antibody; other antibodies such as those described herein are useful.

The MAOs referred to hereinbelow are of human origin. Purified monoamine oxidase A (MAO-A) is isolated from the membranes of human placental mitochondria by published methods. (Weyler, W. and

Salach, J.I [1985]; *J. Biological Chemistry*, **260**:13199
- 13207). As a source of monoamine oxidase B (MAO-B),
the membranes of human platelets are prepared by the
method of Fritz (Fritz, R.R., Abell, C.W., Denney, R.M.
5 et al [1986]; *Psychiatry Res* **17**:129-140) and pure MAO-B
is obtained according to the methods described by Salach
(Salach J.I. [1979]; *Arch. Biochem. Biophys.* **192**:128-
137, & Weyler W., and Salach, J.I. [1981]; *Arch.*
Biochem. Biophys. **212**:147-153).

10 Samples of both enzyme types are further purified
on an immunoaffinity column using an immobilized anti-
MAO-B monoclonal antibody (Denny R.M., Fritz R.R., Patel
N.T. and Abell C.W. [1982]; *Science* **215**:1400-1403) to
ensure complete separation of MAO-A from MAO-B.

15 The cDNAs encoding human MAO-A and MAO-B are
obtained according to published methods (Bach A.W., Lan
N.C., Johnson D.L. et al [1988]; *Proc. Natl. Acad. Sci.*
USA. **85**:4934-4934) and expressed in a CosII cell line
(Lan N.C., Heinzmann C., Klisak I., et al [1988]; *Abst.*
20 *Soc. Neurosci.* **14**:317) or is produced as a recombinant
product from the cloned MAO genes which are overexpressed
in *Escherichia coli* (E.coli). Purification from these
sources is carried out as described above.

Example 11

(11a) The Reaction of an Antibody with Sulfo-SMCC;
Preparation of ING-1-Maleimide (formation of Z-(L₁))

5

A sulfo-SMCC solution (36 nmoles; Pierce Chemical Co.) in phosphate buffered saline (PBS) is added to a solution containing of a chimeric antibody (ING-1; 6 nmoles) in phosphate buffer (at pH 7). The resulting reaction mixture is allowed to stand for 30 minutes with occasional mixing at room temperature. The reaction is stopped with 60 nmoles of basic tris buffer. The reaction mixture is diluted with phosphate buffered saline, added to a prewashed PD-10 column, and eluted with PBS to afford ING-1-maleimide. This material is stored on ice until use.

10

15

(11b) Reaction of Antibody with 2-Iminothiolane;
Preparation of a Mercaptoalkyl-ING-1, ING-1-
(NH)C(=NH₂⁺)CH₂CH₂CH₂SH, a Mercaptoalkyl-antibody
(formation of Z-(L₁)).

20

A solution containing a chimeric antibody (ING-1; 6 nmoles) in 0.1 M carbonate buffer (pH 8.8) is mixed with 200 nmoles of an aqueous solution of 2-iminothiolane. The resulting mixture is allowed to stand for 30 minutes with occasional mixing at room temperature. The reaction

25

mixture is diluted with phosphate buffed saline, added directly to a prewashed PD-10 column, and eluted with PBS to afford mercaptoalkyl-ING-1. This material is stored on ice until use.

5

(11c) The Reaction of an Antibody with SATA;
Preparation of ING-1(NH)-CO-CH₂-SH, a mercapto-antibody (formation of Z-(L₁)).

10

An solution containing 6 nmoles of ING-1 in PBS is vortexed while 60 nmoles of SATA (Pierce Chemical Co), dissolved in DMSO, are added. After mixing and standing at room temperature for 60 min, the reaction mixture is diluted with PBS and eluted from a PD-10 column with PBS

15

to afford the S-acetyl thioacetylated antibody, ING-1(NH)-CO-CH₂-S-CO-CH₃. This S-acetyl thioacetylated antibody is deacylated by the addition of 30 mL of a pH 7.5 solution containing 100 mM sodium phosphate, 25 mM EDTA, and 50 mM NH₂OH. The reaction proceeds for two

20

hours at room temperature after which the material is passed down a PD-10 column eluting with PBS. The final product, ING-1(NH)-CO-CH₂-SH, is used immediately.

25

(11d) Radioisotopic labeling of ING-1 with ^{125}I ; the formation of ^{125}I -labeled ING-1

5 An aliquot of a solution of ING-1 (500 mg) is treated with ^{125}I monochloride (at about 5 mCi/mg) in the presence of Iodogen (sodium N-chlorobenzenesulfonamide) beads in a volume of 500 mL of 100 mM phosphate buffer at pH 7.2 and at room temperature. After 15 minutes the reaction is terminated by passage of the labeled
10 antibody down a prewashed NAP-5 column. The iodinated protein is eluted with PBS and stored at 4°C until use.

Example 12

15 (12a) The Reaction of MAO using SATA; Preparation of a mercapto-MAO (formation of (L₁-Rec))

A solution containing 50 nmoles of MAO in PBS is vortexed while 500 nmoles of SATA (in DMSO) are added.
20 After mixing and standing at room temperature for 60 min, the reaction mixture is diluted with PBS, and eluted from a PD-10 column with PBS to afford an S-acetyl thioacetylated MAO, $\text{MAO}(\text{NH})-\text{CO}-\text{CH}_2-\text{S}-\text{CO}-\text{CH}_3$. The S-acetyl thioacetylated MAO is deacylated by the
25 addition of 25 mL of a pH 7.5 solution containing 100 mM sodium phosphate, 25 mM EDTA, and 100 mM NH_2OH . The reaction proceeds for two hours at room temperature

after which the material is passed down a PD-10 column eluting with PBS. The final product, MAO(NH)-CO-CH₂-SH, is used immediately.

- 5 (12b) Reaction of MAO with 2-Iminothiolane; Preparation of a mercaptoalkyl-MAO, MAO(NH)C(=NH₂⁺)CH₂CH₂CH₂SH . . . (formation of (L₁-Rec))

10 A sample of MAO (50 nmoles) is dissolved in 0.1 M carbonate buffer (pH 9), and 4 mmoles of an aqueous solution of 2-iminothiolane (Pierce Chemical Co) are added. The reactants are vortex mixed and kept at room temperature for 120 minutes. The reaction mixture is quenched by the addition of 4 mmoles of ethanolamine
15 diluted with phosphate buffed saline. The reaction mixture is then added to a prewashed PD-10 column, and eluted with PBS to afford MAO(NH)C(=NH₂⁺)CH₂CH₂CH₂SH. When used for conjugation to ING-1-Maleimide, the product is eluted off the column directly into the ING-1
20 solution.

- (12c) Reaction of MAO with Sulfo-SMCC for the preparation of MAO-Maleimide (formation of (L₁-Rec)).

25 A solution of sulfo-SMCC (300 nmoles: Pierce Chemical Co) in PBS is added to a sample of MAO (50 nmoles) in phosphate buffer (pH 7). The resulting

reaction mixture is allowed to stand for 30 min with occasional mixing at room temperature. The reaction is stopped with 60 nmoles basic tris buffer. The reaction mixture is then diluted with phosphate buffed saline, added to a prewashed PD-10 column, and eluted with PBS to afford MAO-maleimide. This material is stored on ice until use.

(12d) Radioisotopic labeling of MAO with ^{125}I ; the formation of ^{125}I -labeled MAO

An aliquot of MAO (500 mg) is treated with ^{125}I monochloride (at about 5 mCi/mg) in the presence of Iodogen (Sodium N-chlorobenzenesulfonamide) beads in a volume of 500 mL of 100 mM phosphate buffer (pH 7.2) at room temperature. After 15 minutes the reaction is terminated by passage of the thus labeled protein down a prewashed NAP-5 column. The iodinated MAO is eluted with PBS and stored at 4°C until use.

Example 13. General method for the conjugation of a MAO to an Antibody employing the reaction of a sulfhydryl-containing species with a maleimide-containing species, (formation of Z-(L₁-Rec)_n)

The following procedure is generally applicable to the conjugation of sulfhydryl-containing MAO species to

maleimide-containing antibody species as well as to the conjugation of sulfhydryl-containing antibody species to maleimide-containing MAO species. In particular, the following procedure is applicable to the conjugation of

5 ING-1-Maleimide of Example 11a to mercapto-MAO of Example 12a; to the conjugation of ING-1-Maleimide of Example 11a to MAO(NH)C(=NH₂⁺)CH₂CH₂CH₂SH of Example 12b; to the conjugation of materials of ING-1-(NH)C(=NH₂⁺)CH₂CH₂CH₂SH of Example 11b to the MAO-

10 Maleimide of Example 12c; to the conjugation of ING-1-(NH)-CO-CH₂-SH of Example 11c to the MAO-Maleimide of Example 12c.

In this general procedure, the molar ratio of sulfhydryl-containing reactant species (for example,

15 mercapto-MAO of Example 12a; MAO(NH)C(=NH₂⁺)CH₂CH₂CH₂SH of Example 12b; ING-1-(NH)C(=NH₂⁺)CH₂CH₂CH₂SH of Example 11b; and ING-1-(NH)-CO-CH₂-SH of Example 11c) and maleimide-containing reactant species (for example, ING-1-Maleimide of Example 11a and MAO-Maleimide of Example

20 12c) is maintained at a constant value during the conjugation in order to minimize over-conjugation between the proteins.

A freshly prepared sample of a sulfhydryl-containing reactant species as described above (50

25 nmoles) is eluted off a PD-10 column directly into a solution of maleimide-containing species as described above (5 nmoles). After a brief mixing the solution is

rapidly concentrated by centrifugation in a Centricon-30[®] device to a concentration of approximately 3.0 mg/mL protein. The reaction then is allowed to proceed for 4 hours at room temperature. The thus prepared antibody-MAO conjugate is transferred to an Amicon stirred cell fitted with a YM-100 membrane filter, the sample is diluted to 10 mL with PBS and then concentrated, under a nitrogen pressure of 5 kg/cm², to a volume of about 500 microliters. The retentate material is again diluted with PBS to 10 mL and reconcentrated to 1.0 mL. This procedure, which separates unconjugated MAO and other low molecular weight species from the retained antibody-MAO conjugate and unconjugated antibody, is repeated 4 times or until spectrophotometric monitoring of the diafiltrate at 280 nm shows that no further protein is present in the diafiltrate. The retentate material is then concentrated to approximately 1.0 mg of antibody-MAO conjugate per milliliter solution. This solution is then applied to a 2.6 x 60 cm Sephacryl S-200 (Pharmacia) size-exclusion column equilibrated and eluted with a 50 mM sodium phosphate buffer solution at pH 7.2 supplemented with 150 mM sodium chloride. This column separates unconjugated antibody from antibody-MAO conjugate. Fractions of the eluate containing the conjugate are pooled and then centrifuged in a Centricon-30 device to a concentration of approximately 1.0 mg of antibody-MAO conjugate per milliliter of

solution. The solution of the conjugate is sterile filtered through a 0.22 m filter and stored at 4°C until used.

Addition of known trace amounts of either ^{125}I -labeled MAO or ^{125}I -labeled ING-1 to the reaction mixture permits the ratio of one protein to the other after conjugation to be determined by MAO activity and ING-1 assay methods, respectively.

The following examples relate to System B, i.e.,

SYSTEM B

	Non-Radioactive Targeting ImmunoReagent NRTIR	Radioactive Delivery Agent RDA
1	Immunoreactive group + (linking group + ligand) _n	Receptor + (linking group + chelating agent + radionuclide) _m
2	Z-(L ₁ -D) _n	Rec-(L ₂ -Q-M) _m
3	Antibody-(L ₁ -Pargyline) _n	MAO-(L ₂ -TMT- ^{90}Y) _m

wherein:

Z is the residue of an immunoreactive group, preferably an antibody;

Rec is the residue of a receptor, preferably a MAO receptor;

D is the residue of a ligand, preferably a pargyline-containing ligand, that has an affinity for covalent binding to the receptor, preferably to a MAO receptor; L₁ and L₂ are each independently the residue of a linking group that may contain a spacing group; Q is the residue of a chelating group, preferably TMT; M is a radionuclide, preferably ⁹⁰Y; and n and m are each independently an integer greater than zero.

10

Example 14

Synthesis of Fmoc-HN-Ala-Ala-Ala-Ala-OH (SEQ ID NO:10)

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The linear N-alpha-Fmoc-protected peptide, Fmoc-HN-Ala-Ala-Ala-Ala-OH (SEQ ID NO:10), is synthesized via solid-phase methodology on an ABI 430A Automated Peptide Synthesizer. The solid support used in the synthesis is a 4-alkoxybenzyl alcohol polystyrene resin (Wang resin). The N-alpha-Fmoc protecting group is used throughout the synthesis. The peptide chain is assembled using the ABI FastMoc™ software protocols for Fmoc-chemistry (0.25 mmole scale, HBTU activated couplings, 4 fold excess of amino acid, 1 hour). The peptide is removed from the resin by treatment of the peptide-resin with 15 mL of a 95:5 solution of trifluoroacetic acid in water in a sealed vessel followed by shaking at room temperature for 2 hours. The mixture is then filtered using a

20

25

scintered glass funnel. The filtrate volume is then reduced to about 3 mL by rotoevaporation, and the peptide is precipitated by dropping the oil into a centrifuge tube containing 50 mL of ether. The peptide is separated by centrifugation, the ether is decanted, the solid is washed with more ether and then allowed to air dry.

Example 15

Synthesis of H₂N-Ala-Ala-Ala-Ala-OH (SEQ ID NO:11)

The N-alpha-Fmoc-protected peptide, Fmoc-HN-Ala-Ala-Ala-Ala-OH (SEQ ID NO:10), from Example 14 (10 mM) is dissolved in 50 mL of deionized water and 50 mL of dimethylsulfoxide, and then treated with 25 mM of N,N-diethylamine. The reaction vessel is sealed and warmed to 40 °C for 12 hours. The reaction is cooled, and the desired product is isolated by HPLC using a Shodex WS-803F size exclusion column and a UV-visible detector monitoring the absorption of the peptide. A solid sample is isolated from solution by lyophilization.

Example 16

Synthesis of Fmoc-HN-(CH₂)₅-CO-HN-Ala-Ala-Ala-Ala-OH
(SEQ ID NO:12)

5

The crude reaction product from Example 5 is treated with 10 mM of H₂N-Ala-Ala-Ala-Ala-OH (SEQ ID NO:11) from Example 15 in 50 mL of deionized water and 50 mL of dimethylsulfoxide containing 1 mL of pyridine.

10

The reaction is allowed to proceed overnight at room temperature.

Example 17

15

Synthesis of H₂N-(CH₂)₅-CO-HN-Ala-Ala-Ala-Ala-OH (SEQ ID NO:13)

The crude reaction product from Example 16 is treated with diethylamine according to the procedure of Example

20

3. The desired product is purified by HPLC using a Shodex WS-803F size exclusion column and a UV-visible detector to monitor the peptide. A solid sample is isolated from solution by lyophilization.

25

Example 18

Synthesis of $\text{Br-CH}_2\text{-C}_6\text{H}_6\text{-CH}_2\text{-CO-HN-(CH}_2\text{)}_5\text{-CO-HN-(Ala)}_4\text{-OH}$
(SEQ ID NO:14)

5

A solution of $\text{H}_2\text{N-(CH}_2\text{)}_5\text{-CO-HN-Ala-Ala-Ala-Ala-OH}$ (SEQ ID NO:13; 1 mM) from Example 17 in 50 mL of deionized water and 50 mL of DMSO is saturated with sodium carbonate in an ice bath. The peptide is treated with a solution of
10 4-bromomethylphenylacetyl chloride (2 mM) in
20 mL of tetrahydrofuran. The reaction is stirred at ice temperature for 30 min and used immediately.

Example 19

15

Synthesis of $\text{H-CC-CH}_2\text{-N(CH}_3\text{)-CH}_2\text{-C}_6\text{H}_6\text{-CH}_2\text{-CO-HN-(CH}_2\text{)}_5\text{-CO-HN-(Ala)}_4\text{-OH}$ (SEQ ID NO:15) (formation of (L₁-D)

20

To the crude reaction product of Example 18 is added 10 mL of N-methylpropargylamine (Aldrich), and the reaction mixture is stirred for 6 hours at room temperature. The desired product is isolated by HPLC using a Shodex WS-803F size exclusion column and a UV-visible detector monitoring the absorption of the aromatic species. A
25 solid sample is isolated from solution by lyophilization.

Example 20

Synthesis of $\text{H-CC-CH}_2\text{-N(CH}_3\text{)-CH}_2\text{-C}_6\text{H}_6\text{-CH}_2\text{-CO-HN-(CH}_2\text{)}_5\text{-CO-HN-(Ala)}_3\text{-NH-CH(CH}_3\text{)-C(=O)-O-C(=N-C}_2\text{H}_5\text{)-HN-(CH}_2\text{)}_3\text{-HN}^+\text{-(CH}_3\text{)}_2$.

The product of Example 19 (12 nmoles) in 2.5 mL of 50% DMSO in 50 mM sodium phosphate buffer at pH 7.2 is mixed with a solution of EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) in DMSO to give a final EDC concentration of 50 micromolar. After 1 hour at room temperature, the O-acylisourea derivative of Example 19 is isolated by elution from a PD-10 (Pharmacia) size exclusion column with 3.0 ml of 200 mM acetate buffer (pH 5.0). The unreacted EDC is retained and the desired material, hereinafter referred to as the pargylinyl tetrapeptide-O-acylisourea, is used immediately by elution directly into an antibody solution.

Example 21

Conjugation of Pargylinyl tetrapeptide-O-acylisourea to ING-1 (formation of Z-L₁-D).

The pargylinyl tetrapeptide-O-acylisourea obtained from Example 20 (10 nmoles) is eluted directly into a solution of 10 nmoles of ING-1 antibody in 200 mM

acetate buffer (pH 5.0). The reaction mixture is slowly stirred overnight at room temperature. The thus produced pargylinyl tetrapeptide-ING-1 conjugate is separated from unconjugated pargylinyl tetrapeptide-O-acylisourea and other low molecular weight products using a Superose 6 HPLC column equilibrated in and eluted with 50 mM sodium phosphate buffer at pH 7.2 supplemented with 150 mM sodium chloride. The eluate is concentrated using a Centricon-30 device to a concentration of 1.0 mg pargyline tetrapeptide-ING-1 conjugate per milliliter of solution.

Example 22

Conjugation of Monoamine Oxidase to chelating agent, TMT-isothiocyanate (formation of REC-L₂-Q, a MAO-to-TMT conjugate)

A solution of monoamine oxidase (50 nmoles) in 1 mL of 1.0 M carbonate buffer containing 150 mM sodium chloride at pH 9.3 in an acid washed, conical, glass reaction vial is treated with 250 nmoles of the chelating agent, tetrasodium 4'-(3-isothiocyanato-4-methoxyphenyl)-6,6"-bis[N,N-di(carboxymethyl)aminomethyl]-2,2':6',2"-terpyridine, hereinafter referred to as TMT-isothiocyanate which is disclosed in WO 92/08494 (PCT/US91/08253). The reaction mixture is stirred briefly to mix the reactants and then

left in the dark at room temperature. After 16 hours, the therein produced MAO-to-TMT conjugate is separated from unconjugated TMT-isothiocyanate by applying the reaction mixture to a PD-10 chromatography column which has been pre-washed and equilibrated with 50 mM sodium acetate buffer containing 150 mM sodium chloride at pH 5.6. The conjugate is eluted off the column with 2.5 mL of that same buffer, and concentrated on a Centricon-10[®] concentration device.

Example 23

Radioisotopic labeling of MAO-to-TMT conjugate with ⁹⁰Y: (formation of Rec-(L₂-Q-M)_m)

A volume of radioactive yttrium chloride (⁹⁰Y in 0.04 M hydrochloric acid at a specific activity of >500 Ci/g: Amersham-Medipysics) is neutralized using two volumes of 0.5 M sodium acetate pH 6.0. The neutralized ⁹⁰Y solution (1.0 mCi) is added to 1.0 mL of MAO-to-TMT conjugate (1 mg/mL) in 50 mM sodium acetate buffer containing 150 mM sodium chloride at pH 5.6. After one hour the reaction mixture is loaded on to a PD-10 chromatography column which has been pre-washed with and equilibrated in a pH 7.4 phosphate buffer containing 50 mM sodium phosphate and 150 mM sodium chloride (PBS). The sample is eluted from the column with 1.5 mL of PBS. Fractions of radioisotopically labeled MAO-to-TMT

conjugate (0.5 mL) are collected, assayed for radioactivity, and pooled. The labeling efficiency is determined by removing 1.0 μ L of the sample and spotting it on to a Gelman ITLC-SG strip. The strip is developed in a glass beaker containing 0.1 M sodium citrate, pH 6.0, for a few minutes until the solvent front has reached three-quarters of the way to the top of the paper. The strip is inserted into a System 200 Imaging Scanner (Bioscan) which is optimized for ^{90}Y and controlled by a Compaq 386/20e computer. In this system free ^{90}Y migrates at the solvent front while the MAO-to-TMT conjugate isotopically labeled with ^{90}Y remains at the origin. Using this system more than 98% of the total ^{90}Y radioactivity is found associated with MAO-to-TMT conjugate at the origin.

Example 24 Assays on the MAO-containing conjugates prepared from System A or System B

(24a) Protein Concentration

The concentrations of ING-1 and MAO for use in the conjugate reactions are determined by the BioRad protein assay using bovine immunoglobulin as the protein standard. By inclusion of known trace amounts of ^{125}I -labeled MAO or of ^{125}I -labeled ING-1 (the amount of radioactivity being measured by liquid scintillation

counting) in the reaction mixtures, and by knowing the specific activity of the proteins in the preparations, the ratio of one protein to the other after conjugation is calculated.

5 As an alternative to using trace amounts of radiolabeled ^{125}I -MAO, MAO labeled with other materials, such as TMT-isothiocyanate which is then chelated to ^{90}Y as described above can be used. The amount of radioactivity can be measure as above. Alternatively,
10 MAO labeled with a chelating agent such as TMT-isothiocyanate as described above can be chelated to europium ion for use in fluorescence detection assays. Other known assays for quantifying concentrations of proteins such as those involving biotinylating agents
15 such as those described in the Pierce Chemical Company 1992 catalog, or assays involving fluorescein isothiocyanate are useful to detect and quantify the amount of MAO or MAO conjugated to another protein and
20 present in a solution. Such species include MAO conjugated to an antibody, sometimes hereinafter referred to as antibody-MAO conjugates.

(24b) Immunoreactivity assay by Flow Cytometry

25 Antibody-MAO conjugates are examined for their ability to bind to antigens on the surface of a human tumor cell line to which the antibody had been raised.

The immunoreactivity of the conjugates is compared by flow cytometry with a standard preparation of the antibody before being subjected to modification and conjugation to MAO. Target HT29 cells (a human adenocarcinoma cell line: ATCC) are grown to confluency in tissue culture flasks using McCoy's media supplemented with 10% fetal calf serum. The cells are harvested by scraping the flask walls with a cell scraper. Cells from many separate flasks are pooled, centrifuged to a pellet, resuspended at 5×10^5 /mL in a solution of ice-cold 50 mM sodium phosphate with 150 mM sodium chloride buffer pH 7.4 (PBS) supplemented with 0.1% bovine serum albumin (Sigma) and 0.02% sodium azide (Flow buffer). The cells are washed in this same buffer and then counted. An antibody standard curve is constructed by diluting a stock solution of ING-1 with an irrelevant (non binding), isotype-matched control antibody (human IgG1) to give a number of samples ranging in ING-1 content from 10% to 100%. The standard curve is made in flow buffer so that each sample contains 1.0 mg protein per mL. Samples from the standard curve and ING-1-MAO unknowns are then incubated with 5×10^5 HT29 cells at 4°C for 1 hour. After extensive washing to remove unbound antibody, the cells are resuspended in 100 mL flow buffer and incubated at 4°C for 1 hour with goat-anti-human antibody labelled with fluorescein isothiocyanate (FITC). After further

washing in flow buffer the samples are analyzed by flow cytometry on a Coulter EPICS 753 flow cytometer. Fluorescein isothiocyanate (FITC) and propidium iodide (PI) are excited using the 488 nm emission line of an argon laser. The output is set at 500 mw in light regulation mode. Single cells are identified by 90 degree and forward angle light scatter. Analysis windows are applied to these parameters to separate single cells from aggregates and cell debris. Fluorescence from FITC and propidium are separated with a 550nm long pass dichroic filter and collected through a 530 nm band pass filter (for FITC), and a 635 nm band pass filter (for PI). Light scatter parameters are collected as integrated pulses and fluorescence is collected as log integrated pulses. Dead cells are excluded from the assay by placing an analysis window on cells negative for PI uptake. The mean fluorescence per sample (weighted average from 2500 cells) is calculated for each histogram. FITC calibration beads are analyzed in each experiment to establish a fluorescence standard curve. The average fluorescence intensity for each sample is then expressed as the average FITC equivalents per cell. Immunoreactivity is calculated by comparing the average fluorescence intensity of the ING-1-MAO sample with values from the standard curve.

(24c) Immunoreactivity assay by ELISA

The antigen to which the antibody, ING-1, binds is prepared from LS174T or HT 29 cells (available from
5 ATTC) by scraping confluent monolayers of cells from the walls of culture flasks with a cell scraper. The cells from many flasks are combined and a sample is taken and counted to estimate the total number of cells harvested. At all times the cells are kept on ice. Following
10 centrifugation of the cells at 1500 rpm for 10 minutes at 4°C , the cells are washed once in 25 mL ice-cold 50 mM sodium phosphate buffer, pH 7.4 supplemented with 150 mM sodium chloride (PBS), pelleted under the same conditions and transferred in 10 mL PBS to an ice-cold
15 glass mortar. The cells are homogenized at 4°C using a motor-driven pestle and then centrifuged at 3000 x g for 5 minutes. The antigen-rich supernatant is removed from the other cell debris and subjected to further centrifugation at 100,000 x g for one hour at 4°C. The
20 pellet (antigen fraction) from this final step is suspended in 100 mL of PBS for every million cells harvested. Following an estimate of the protein concentration (BioRad BCA protein assay using bovine immunoglobulin as the protein standard) the antigen is
25 stored at at -20°C until use.

Each well of a 96-well Costar microtiter plates is coated with antigen by adding 100 µL/well of cell lysate

(10 mg/ml) prepared as above. The microtiter plates are allowed to dry overnight in a 37°C incubator. After washing the plate five times with 0.05% Tween-20 (Sigma) they were blotted dry. The wells of each plate were
5 blocked by adding 125 mL/well of a 1% BSA (bovine serum albumin, Sigma A-7906) solution in PBS and incubated for 1 hour at room temperature. The plates were washed five times with 0.05% Tween-20. Samples (50 mL/well in duplicate) of ING-MAO conjugates and standard ING-1
10 antibody solutions were prepared at a range of concentrations in 1% BSA in PBS. Biotinylated ING-1 (1.0 mg/mL in 0.1% BSA) is added to each well (50 mL/well) and the plates are then incubated for 2 hours at room temperature. Following five washes with 0.05%
15 Tween-20, the plates are blotted dry and incubated at room temperature for one hour with dilute (1:2000 in 0.1% BSA) streptavidin-alkaline phosphatase (Tago; #6567). After a further five washes, color was developed in each well upon the addition of 100 mL per
20 well of phosphatase substrate reagent (two Sigma 104 phosphatase tablets dissolved in 10 mL distilled water and 20 mL Sigma 221 alkaline buffer). After one hour at room temperature, the color was read using a 405 nm filter in a Titertek Multiscan microplate reader.

25

(24d) SDS PAGE gel electrophoresis

5 Samples of conjugates are subjected to electrophoresis on Novex 4%-20% reduced and native polyacrylamide gels using SDS buffers to estimate their apparent molecular weight and the degree of heterogeneity of the preparation. Using standards of known molecular weight run on the same gel, a standard curve is constructed of the distance travelled (Rf) versus the log of the molecular weight. From this standard curve the relative molecular weights of the bands associated with each conjugate preparation are determined.

15 (24e) Determination of aggregate formation by size-exclusion HPLC.

20 A 30 cm x 7.5 mm TSK-G3000SW size-exclusion HPLC column (Supelco) fitted with a guard column of the same material is equilibrated with 12 column volumes of 10 mM sodium phosphate buffer pH 6.0 supplemented with 150 mM sodium chloride using a Waters 600E HPLC system with a flow rate of 1.0 mL per minute at 400-600 PSI. A sample (25 mL) of BioRad gel filtration protein standards is injected on to the column. The retention time of each standard is monitored by a Waters 490 UV detector set at 280 nm. Following the recovery of the final standard

25

from the column, it is washed with a further 10 volumes of 10 mM sodium phosphate buffer pH 6.0 supplemented with 150 mM sodium chloride. Separated samples (50 microliters) of solutions containing ING-1 antibody, 5 ING-1-MAO conjugate (from Example 13), pargylinyl tetrapeptide-ING-1 conjugate (from Example 21), and MAO-to-TMT conjugate (from Example 22), all at 200 microgram/mL, are injected on to the column and their respective retention times are recorded on a linear 10 response strip chart recorder which displays the elution of the samples as a function of time and which provides a linear correlation recording of sample elution profile as peaks on the chart. From the areas of the peaks and the retention time measured at peak maxima, the amount 15 of aggregated material in the samples is calculated.

(24f) Determination of MAO activity

20 In a manner analogous to well known methods of measuring the binding of the antibody to its antigen, the enzymatic activity of MAO before and after conjugation is assayed to ensure that the process of conjugation of MAO to another species as described above does not inhibit the enzymatic activity of MAO.

25 As another non-limiting example, the enzymatic activity of MAO is used to monitor inhibitory effect of drugs such as clorgyline, pargyline and their analogs as

described above in Examples 1 - 9 on unconjugated MAO and on antibody-MAO conjugates.

As still another non-limiting example, the enzymatic activity of MAO in antibody-MAO conjugates as described above is used as a measure of efficacy of new drugs designed to inhibit MAO activity. It is also used as a measurement of the effect of clorgyline-derived or pargyline-derived TMT delivery systems, D-(L₂-Q)_m as described above, such as H-CC-CH₂-N(CH₃)-CH₂-C₆H₅-CH₂-CO-HN-(CH₂)₅-CO-HN-(Ala)₄-(Lys-TMT)₂-OH (SEQ ID NO:7) of Example 9 as well as those systems that contain a chelated metal ion, D-(L₂-Q-M)_m, such as described above for ⁹⁰Y.

In yet another non-limiting example, the enzymatic activity of MAO is used to as a measure of the relative amount of MAO in a solution.

1 unit of MAO activity is defined as the amount of material needed to convert 1.0 micromole of kynurine to 4-hydroxyquinoline per minute at pH 7.2 and 25°C. The activity of the enzyme is measured spectrophotometrically at 314 nm by following the increase in absorbance as kynurine is oxidized to 4-hydroxyquinoline.

To start the analysis, a sample of purified MAO enzyme (approximately 1.0 mg) or a sample of ING-1-MAO conjugate (Example 13) or a sample of MAO-to-TMT conjugate (Example 22) is added to a 1.0 mM solution of

kynurine dissolved in a 50 mM phosphate buffer (pH 7.2) containing 0.2% Triton X-100 at 30°C to give a final volume of 1.0 mL. The increase in absorbance at 314 nm is measured over a 10 minute period and the activity (units per mL) is calculated from the slope of the optical density vs time plot (Weyler, W. and Salach, J.I [1985]; *J. Biological Chemistry*, 260:13199 - 13207).

In order to measure the effect of inhibitors (e.g. Examples 9, 19 or 21) on the activity of the enzyme, increasing concentrations of the inhibitor are added into the basic MAO enzyme assay, described above, without changing the volume or concentration of the reactants. The concentration of inhibitor required to reduce the amount of 4-hydroxyquinoline produced by 50% is calculated and compared with known concentrations of pargyline.

Such calculations measure the inhibitor's effects on enzyme activity, but to calculate the total number of binding sites available to an inhibitor, MAO-containing samples are incubated at 30°C for 3 hours with 5 micromolar ³H-pargyline (22.5 Ci/mmol: New England Nuclear) dissolved in 0.05 M phosphate buffer (pH 7.2) in the absence or presence of varying amounts of the inhibitors described herein (e.g., Examples 9, 19 and 21). The quantity of ³H-pargyline bound to MAO is determined by immunoprecipitation of the radiolabeled enzyme by using anti-MAO-A or anti-MAO-B monoclonal

antibodies (Riley, L.A., Waguespack M.A. and Denney,
R.M. [1989]; *Molecular Pharmacology* 36:54-60). A
comparison of the total available binding sites with
enzyme activity in a particular sample gives a measure
5 of protein degradation during conjugation procedures.

The present invention has been described in detail with
particular reference to certain preferred embodiments
thereof, but it will be understood that variations and
10 modifications can be effected within the spirit and
scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- 5 (i) APPLICANT: Black, Christopher D. V.
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- 10 (ii) TITLE OF INVENTION: IMMUNOREACTIVE AGENTS
EMPLOYING MONOAMINE OXIDASE
- (iii) NUMBER OF SEQUENCES: 15
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25 (A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version
#1.25
- 30 (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:
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- 45 (2) INFORMATION FOR SEQ ID NO:1:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
50 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

5 Ala Ala Ala Ala Lys Lys
 1 5

(2) INFORMATION FOR SEQ ID NO:2:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 6 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

20 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 1
 (D) OTHER INFORMATION: /note= "X represents
 FMOC protected Ala."

25 (ix) FEATURE:
 (A) NAME/KEY: Peptide
 (B) LOCATION: 1..6
 (D) OTHER INFORMATION: /note= "The Lys
 residues are bis(t-BOC)-blocked; the
 entire peptide is resin bound."

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

35 Xaa Ala Ala Ala Lys Lys
 1 5

(2) INFORMATION FOR SEQ ID NO:3:

40 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 6 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: peptide

50 (ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 1
 (D) OTHER INFORMATION: /note= "X represents
 FMOC-Ala."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Xaa Ala Ala Ala Lys Lys
1 5

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
(B) LOCATION: 5..6
(D) OTHER INFORMATION: /note= "Each X
represents Lys-TMT."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ala Ala Ala Ala Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "X represents
FMOC-Ala."

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
(B) LOCATION: 5..6
(D) OTHER INFORMATION: /note= "X represents
Lys-TMT."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Xaa Ala Ala Ala Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "X represents
FMOC-HN-(CH₂)₅-CO-."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 6..7
(D) OTHER INFORMATION: /note= "X represents
Lys-TMT."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Xaa Ala Ala Ala Ala Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "X represents
H₂N-(CH₂)₅-CO-."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 6..7
(D) OTHER INFORMATION: /note= "X represents
Lys-TMT."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Xaa Ala Ala Ala Ala Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:8:

5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

10 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "X represents
15 Br-CH2-C6H6-CH2-CO-HN- (CH2) 5-CO-."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 6..7
(D) OTHER INFORMATION: /note= "X represents
20 Lys-TMT."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

25 Xaa Ala Ala Ala Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:9:

30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
40 (B) LOCATION: 1
(D) OTHER INFORMATION: /note= "X represents
H-CC-CH2-N(CH3)-CH2-C6H6-CH2-CO-HN-
(CH2) 5-CO-."

45 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 6..7
(D) OTHER INFORMATION: /note= "X represents
Lys-TMT."

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Xaa Ala Ala Ala Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "X represents
FMOC-Ala."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Xaa Ala Ala Ala
1

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ala Ala Ala Ala
1

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "X represents
FMOC-HN-(CH2)5-CO-."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

5 Xaa Ala Ala Ala Ala
1 5

(2) INFORMATION FOR SEQ ID NO:13:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
20 (D) OTHER INFORMATION: /note= "X represents
H2N-(CH2)5-CO-."

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Xaa Ala Ala Ala Ala
1 5

30 (2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
35 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

40 (ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "X represents
Br-CH2-C6H6-CH2-CO-HN-(CH2)5-CO-."

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Xaa Ala Ala Ala Ala
1 5

50 (2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: peptide

(ix) FEATURE:

10 (A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "X represents
H-CC-CH2-N(CH3)-CH2-C6H6-CH2-CO-HN-
(CH2)5-CO-."

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Xaa Ala Ala Ala Ala
1 5

We claim:

1. A targeting immune reagent that comprises moieties represented by the structure:



wherein:

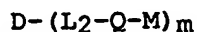
Z comprises the residue of an immunoreactive protein;

10 L₁ is a chemical bond or a linking group that may contain a spacing group;

X is the residue of a monoamine oxidase or a ligand specific for a monoamine oxidase; and

n is an integer greater than zero.

15 2. A radioactive targeting reagent comprising moieties represented by the structure:



wherein:

20 D is the residue of a monoamine oxidase or a ligand which will bind a monoamine oxidase;

L₂ is a chemical bond or a linking group that may contain a spacing group;

Q is the residue of a chelating group;

M is a radionuclide; and

25 m is an integer greater than zero.

3. The reagent of claim 1 wherein Z is an antibody or antibody fragment.

5 4. The reagent of claim 1 wherein the antibody is ING-1.

5. The reagent of claim 1 wherein X is the residue of monoamine oxidase-A.

10 6. The reagent of claim 5 wherein the residue of monoamine oxidase is derived from human placenta or as a recombinant protein.

15 7. The reagent of claim 1 wherein X is the residue of monoamine oxidase-B.

8. The reagent of claim 7 wherein the residue of monoamine oxidase-B is derived from human platelets or as a recombinant protein.

20 9. The reagent of claim 3 wherein X is selected from the group consisting of derivatives of clorgyline analogues and derivatives of pargyline analogs.

25 10. The reagent of claim 1 wherein L₁ is a heterobifunctional cross-linking reagent.

11. The reagent of claim 10 wherein the heterobifunctional cross-linking reagent is selected from the group consisting of sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate, sulfosuccinimidyl (4-iodoacetyl)aminobenzoate, sulfosuccinimidyl 4-(p-maleimidophenyl)butyrate, 2-Iminothiolane, and N-succinimidyl S-acetylthioacetate.

12. The reagent of claim 1 wherein L₁ is a modified receptor moiety containing a reactive functional group.

13. The reagent of claim 12 wherein the reactive functional group is selected from the group consisting of amino groups and sulfhydryl groups.

14. The reagent of claim 2 wherein D is selected from the group consisting of derivatives of clorgyline analogues and derivatives of pargyline analogs.

15. The reagent of claim 2 wherein D the residue of monoamine oxidase.

16. The reagent of claim 15 wherein the residue of monoamine oxidase-A is derived from human placenta or as a recombinant protein.

17. The reagent of claim 2 wherein D is the residue of monoamine oxidase-B.

5 18. The reagent of claim 17 wherein said residue of monoamine oxidase-B is derived from human platelets or as a recombinant protein.

10 19. The reagent of claim 2 wherein L₂ is a heterobifunctional cross-linking reagent.

15 20. The reagent of claim 19 wherein the heterobifunctional cross-linking reagent is selected from the group consisting of sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate, sulfosuccinimidyl (4-iodoacetyl)aminobenzoate, sulfosuccinimidyl 4-(p-maleimidophenyl)butyrate, 2-Iminothiolane, and N-succinimidyl S-acetylthioacetate.

20 21. The reagent of claim 2 wherein L₂ is a modified ligand moiety containing a reactive functional group.

25 22. The reagent of claim 21 wherein the reactive functional group is selected from the group consisting of amino groups and sulfhydryl groups.

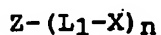
23. The reagent of claim 2 wherein Q contains a polycarboxylic acid group.

5 24. The reagent of claim 2 wherein Q is selected from the group consisting of B4A, P4A, TMT, DCDTPA, PheMT, macroPheMT, and macroTMT.

10 25. The reagent of claim 2 wherein M is a radioactive metal ion isotope.

26. The reagent of claim 25 wherein the radioactive metal ion isotope is ^{90}Y .

15 27. A method of making a compound of the structure:



wherein:

Z comprises the residue of an immunoreactive protein;

20 L_1 is a chemical bond or a linking group that may contain a spacing group;

X is the residue of a monoamine oxidase or the residue of a ligand which will bind a monoamine oxidase; and

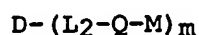
25 n is an integer greater than zero; comprising:

(i) derivatizing L₁ with X under conditions and for a time period sufficient to form a covalent complex L₁-X;

and

(ii) derivatizing Z with L₁-X under conditions and for a time period sufficient to form a covalent complex Z-(L₁-X)_n.

28. A method of making a compound of the structure:



wherein:

D is the residue of a monoamine oxidase or the residue of a ligand which will bind a monoamine oxidase;

L₂ is a chemical bond or a linking group that may contain a spacing group;

Q is the residue of a chelating group;

M is a radionuclide; and

m is an integer greater than zero.

comprising:

(i) derivatizing Q with L₂ under conditions and for a time period sufficient to form a covalent complex Q-L₂;

(ii) derivatizing Q-L₂ with D under conditions and for a time period sufficient to form a covalent complex D-L₂-Q;

(iii) derivatizing D-L₂-Q with M under conditions and for a time period sufficient to form a covalent complex D-(L₂-Q-M)_m.

29. The method of claim 27 wherein Z is an antibody or antibody fragment.

5 30. The antibody of claim 29 wherein the antibody is ING-1.

31. The method of claim 27 wherein X is selected from the group consisting of derivatives of clorgyline
10 analogs and derivatives of pargyline analogs.

32. The method of claim 27 wherein L₁ is a heterobifunctional cross-linking reagent.

15 33. The method of claim 32 wherein the heterobifunctional cross-linking reagent is selected from the group consisting of sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate,
sulfosuccinimidyl (4-iodoacetyl)aminobenzoate,
20 sulfosuccinimidyl 4-(p-maleimidophenyl)butyrate, 2-Iminothiolane, and N-succinimidyl S-acetylthioacetate.

34. The method of claim 27 wherein L₁ is a modified nucleotide moiety containing a reactive
25 functional group.

35. The method of claim 27 wherein the reactive functional group is selected from the group consisting of amine groups and sulfhydryl groups.

5 36. The method of claim 27 wherein X is selected from the group consisting of the residue of monoamine oxidase-A and the residue of monoamine oxidase-B.

10 37. The method of claim 27 wherein the residue of monoamine oxidase is derived from human placenta, human platelets, or as a recombinant protein.

15 38. The method of claim 28 wherein D is selected from the group consisting of derivatives of clorgyline analogues and derivatives of pargyline analogs.

20 39. The method of claim 28 wherein D is the residue of monoamine oxidase.

40. The method of claim 39 wherein the residue of monoamine oxidase is derived from human placenta, human platelets, or as a recombinant protein.

25 41. The method of claim 28 wherein D is the residue of monoamine oxidase-B.

42. The method of claim 41 wherein said residue of monoamine oxidase-B is derived from human platelets, or as a recombinant protein.

5 43. The method of claim 28 wherein L₂ is a heterobifunctional cross-linking reagent.

10 44. The method of claim 43 wherein the heterobifunctional cross-linking reagent is selected from the group consisting of sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate, sulfosuccinimidyl (4-iodoacetyl)aminobenzoate, sulfosuccinimidyl 4-(p-maleimidophenyl)butyrate, 2-Iminothiolane, and N-succinimidyl S-acetylthioacetate.

15 45. The method of claim 28 wherein L₂ is a modified ligand moiety containing a reactive functional group.

20 46. The method of claim 28 wherein the reactive functional group is selected from the group consisting of amine groups and sulfhydryl groups.

25 47. The method of claim 28 wherein Q contains a polycarboxylic acid group.

48. The method of claim 28 wherein Q is selected from the group consisting of B4A, P4A, TMT, DCDTPA, PheMT, macroPheMT, and macroTMT.

5 49. The method of claim 28 wherein M is a radioactive metal ion isotope.

50. The method of claim 49 wherein the radioactive metal ion isotope is ⁹⁰Y.

10

51. A pharmaceutical composition comprising a compound of claim 1 dissolved or dispersed in a pharmaceutically acceptable carrier.

15 52. A pharmaceutical composition comprising a compound of claim 2 dissolved or dispersed in a pharmaceutically acceptable medium.

20 53. A method of treating a tumor in a mammal comprising administering to said mammal an effective dose of a non radioactive targeting immunoreagent of claim 1 in a pharmaceutically acceptable medium, for a time period sufficient for said non radioactive targeting immunoreagent to accumulate at the tumor site
25 in said mammal, and subsequently, administering an effective dose of a radioactive targeting reagent of claim 2 in a pharmaceutically acceptable medium to said

mammal, for a time period sufficient for said
radioactive targeting reagent to accumulate at the
target site, said target site being the said non
radioactive targeting immunoreagent accumulated at said
5 tumor site in said mammal.

54. A method of diagnostic imaging in a mammal
comprising administering to said mammal an imaging
effective dose of a non radioactive targeting
10 immunoreagent of claim 1 in a pharmaceutically
acceptable medium, for a time period sufficient for said
non radioactive targeting immunoreagent to accumulate at
the imaging site in said mammal, and subsequently,
administering an effective dose of a radioactive
15 targeting reagent of claim 2 in a pharmaceutically
acceptable medium to said mammal, for a time period
sufficient for said radioactive targeting reagent to
accumulate at the target site, said target site being
the said non radioactive targeting immunoreagent
20 accumulated at said imaging site in said mammal.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/06424

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : C07K 3/00, 17/06; A61K 39/00, 43/00, 49/00

US CL : 530/391.9, 402; 424/1.69, 9, 179.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/391.9, 402; 424/1.69, 9, 179.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,719,182 (BURDICK ET AL) 12 January 1988, see the various examples.	1-54
Y	US, A, 4,863,713 (GOODWIN ET AL) 05 September 1989, see the SUMMARY OF THE INVENTION, column 7, line 40 and column 9, lines 37-43, in particular.	1-54
Y	US, A, 4,913,891 (FOWLER ET AL) 03 April 1990, see columns 1 and 3 in particular.	1-54
Y,P	US, A, 5,254,329 (KEINER) 19 October 1993, see column 7, line 15, in particular.	1-54
Y	WO, A, 92/08494 (TONER ET AL) 29 May 1992, see the entire document.	1-54



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

23 AUGUST 1994

Date of mailing of the international search report

02 SEP 1994

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